Supporting Information

General and Robust Chemoenzymatic Method for Glycan-Mediated Site-Specific Labeling and Conjugation of Antibodies. Facile Synthesis of Homogeneous Antibody-Drug Conjugates

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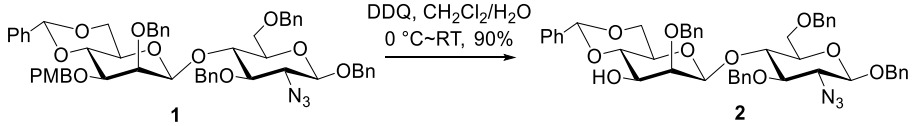
Contents

1. Chemical synthesis of disaccharide oxazolines.

Materials and Methods.

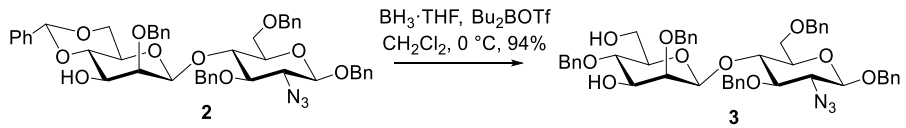
All chemicals, reagents, and solvents were purchased from Sigma−Aldrich and TCI and unless specially noted applied in the reaction without further purification. TLC was performed using silica gel on glass plates (Sigma-Aldrich), and spots were detected under UV light (254 nm) then charring with 5 % (v/v) sulfuric acid in EtOH or cerium molybdate stain (CAM) followed by heating at 150 ºC. Silica gel (200−425 mesh) for flash chromatography was purchased from Sigma-Aldrich. NMR spectra were recorded on a 400 MHz spectrometer (Bruker, Tokyo, Japan) with $CDCl_3$ or D_2O as the solvent. The chemical shifts were assigned in ppm, and multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants (J) are reported in Hertz. MALDI-TOF was performed on a Bruker Autoflex Speed Mass Spectrometer in positive reflectron mode with DHB (ACN/H_2O = 1:1) as the matrix. HRMS was performed on an Exactive Plus Orbitrap Mass Spectrometer (Thermo Scientific) equipped with a C18 column. Preparative HPLC was performed with a Waters 600 HPLC instrument and Waters C18 columns (7.0 μm, 19 × 300 mm). The column was eluted with a suitable gradient of MeCN−H_2O containing 0.1% FA at a flow rate of 10 mL/min. LC-MS analysis was performed on an Ultimate 3000 HPLC system coupled to an Exactive Plus Orbitrap mass spectrometer (Thermo Fischer Scientific) with C4 (whole antibody, gradient, 5−95% aq MeCN containing 0.1% FA for 6 min, 0.4 mL/min) or C8 (IdeS digestion, gradient, 25−35% aq MeCN containing 0.1% FA for 6 min, 0.4 mL/min, or 5−95% aq MeCN containing 0.1% FA for 6 min, 0.4 mL/min) column. Deconvolution data was transformed by MagTran software.

Benzyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (2).



To a solution of **1** (454 mg, 0.485 mmol) in a mixture of CH_2Cl_2/H_2O (15 mL/1 mL) was added DDQ (252 mg, 1.11 mmol) at 0 °C. After 30 min, the reaction mixture was warmed to room temperature and further stirred for 1 h. The reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ (aq.) and brine, and dried over Na_2SO_4. Concentration and purification by column chromatography on silica gel (hexanes/EtOAc = 6:1~3:1) provided **2** (356 mg, 90%) as a white amorphous solid. R_f = 0.25 (hexanes/EtOAc = 3:1); Spectroscopic data were in agreement with literature values. [1] MALDI-TOF: $[M + Na]^+$ calcd for $C_{47}H_{49}N_3NaO_{10}^+$, 838.33; found, 838.47.

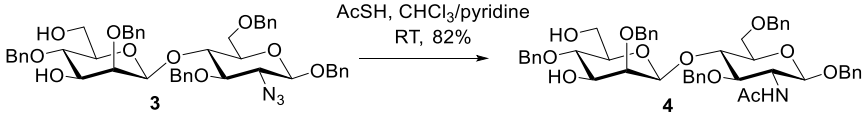
Benzyl 2,4-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (3)



To a solution of compound **2** (197 mg, 0.242 mmol) in BH_3·THF (1 M, 3.0 mL) was added a solution of Bu_2BOTf in CH_2Cl_2 (1 M, 483 μL) under argon atmosphere at 0 °C and the mixture was stirred at

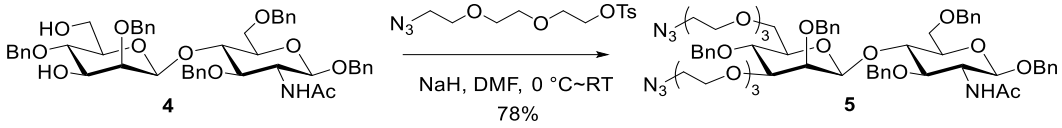
0 °C for 40 min when TLC indicated the completion of the reaction. Et_3N (250 μL) was added to the reaction followed by careful addition of MeOH (500 μL). The mixture was co-evaporated with MeOH three times and the residue was purified by flash chromatography (hexanes/EtOAc = 6:1~2:1) to afford **3** (186 mg, 94%) as a colorless syrup. R_f = 0.30 (hexanes/EtOAc = 2:1); Spectroscopic data were in agreement with literature values. [1] MALDI-TOF: $[M + Na]^+$ calcd for $C_{47}H_{51}N_3NaO_{10}^+$, 840.35; found, 840.32.

Benzyl 2,4-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (4).



A solution of compound **3** (186.3 mg, 0.228 mmol) in a mixture of AcSH/pyridine/CHCl3 (0.8 mL/0.6 mL/0.8 mL) was stirred at room temperature for 20 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/Acetone = 4:1~1:1) to afford compound **4** (154.8 mg, 82%) as colorless syrup. R_f = 0.25 (hexanes/Acetone = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.28 (25H, m, Ar-H), 5.64 (1H, d, J = 7.7 Hz), 5.02-4.95 (3H, m), 4.92 (1H, d, PhCH_2, J = 11.9 Hz), 4.86 (1H, d, PhCH_2, J = 11.2 Hz), 4.68 (1H, d, PhCH_2, J = 12.0 Hz), 4.65-4.57 (5H, m), 4.52 (1H, d, PhCH_2, J = 12.0 Hz), 4.20 (1H, dd, J = 8.5 Hz, J = 8.5 Hz), 3.93 (1H, dd, J = 8.2 Hz, J = 8.2 Hz), 3.83-3.65 (5H, m), 3.56-3.44 (4H, m), 3.14 (1H, m), 2.33 (1H, d, J = 8.7 Hz), 1.98 (1H, m), 1.80 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.52, 138.81, 138.27, 138.09, 137.69, 137.52, 128.59, 128.56, 128.45, 128.39, 128.01, 127.99, 127.96, 127.83, 127.51, 101.26, 98.97, 78.37, 78.12, 77.45, 77.24, 76.54, 75.29, 74.94, 74.69, 74.12, 74.03, 73.67, 70.86, 68.89, 62.23, 56.27, 29.71, 23.49. MALDI-TOF: $[M + Na]^+$ calcd for $C_{49}H_{55}NNaO_{11}^+$, 856.37; found, 856.30.

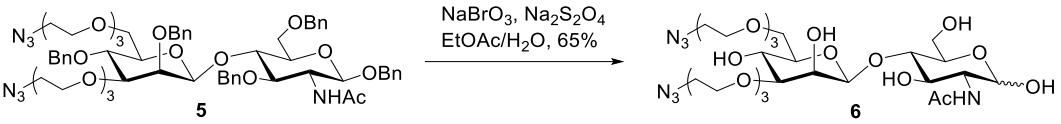
Benzyl 2,4-di-*O*-benzyl-3,6-bis-*O*-2-[2-(2-azidoethoxy)ethoxy]ethyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (5).



To a solution of compound **4** (79.0 mg, 0.095 mmol) and the tosylate linker [2] (125 mg, 0.379 mmol) in anhydrous DMF (3.0 mL) was added 60% sodium hydride (19.0 mg, 0.474 mmol) at 0 °C. After stirring for 0.5 hour at 0 °C then 7.5 hours at room temperature, MeOH (100 μL) and AcOH (25 μL) was added to the reaction mixture at 0 °C. The residual mixture was concentrated to dryness then purified by column chromatography on silica-gel (hexanes/Acetone = 3:1~2:1) to give compound **5** (84.9 mg, 78%) as a colorless syrup. R_f = 0.20 (hexanes/Acetone = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.42 (2H, m, Ar-H), 7.36-7.26 (23H, m, Ar-H), 6.07 (1H, d, J = 8.2 Hz), 4.94-4.79 (6H, m), 4.69-4.65 (6H, m), 4.07 (1H, dd, J = 6.5 Hz, J = 6.5 Hz), 3.95 (1H, dd, J = 5.9 Hz, J = 5.9 Hz), 3.89 (1H, m), 3.86-3.80 (2H, m), 3.79-3.73 (3H, m), 3.66-3.53 (20H, m), 3.52-3.48 (2H, m), 3.32-3.29 (6H, m), 1.69 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.12, 139.00, 138.73, 138.69, 138.05, 137.75, 128.47, 128.34, 128.32, 128.21, 128.18, 127.93, 127.87, 127.84, 127.76, 127.62, 127.59, 127.57, 127.33, 101.11, 99.52, 83.66, 75.73, 75.56, 75.25, 74.82, 74.57, 74.43, 73.50, 73.00, 70.90, 70.67,

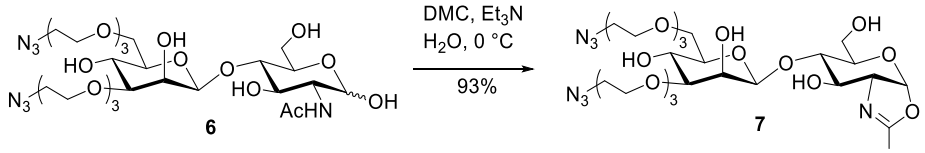
70.60, 70.57, 70.50, 70.45, 70.42, 69.98, 69.91, 69.74, 69.59, 53.24, 50.63, 50.60, 23.12; MALDI-TOF: $[M + Na]^+$ calcd for $C_{61}H_{77}N_7NaO_{15}^+$, 1170.54; found, 1170.70.

3,6-bis-*O*-2-[2-(2-azidoethoxy)ethoxy]ethyl-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (6).



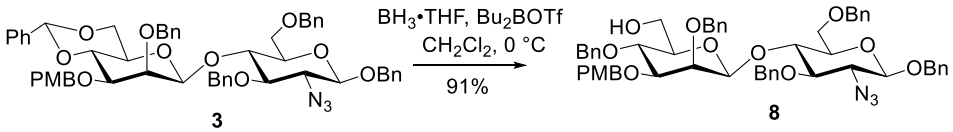
To a solution of compound **5** (83.7 mg, 0.073 mmol) in ethyl acetate (1.2 mL) was added a solution of $NaBrO_3$ (167 mg, 1.09 mmol) in water (0.88 mL). A solution of $Na_2S_2O_4$ (85 %, 194 mg, 0.95 mmol) in water (1.76 mL) was added over 10 min at 0 °C and the mixture was vigorously stirred for 5 h at room temperature. The mixture was quenched with saturated sodium thiosulphate and concentrated in vacuum. The residue was purified by flash chromatography (CH_2Cl_2/MeOH = 10:1~ CH_2Cl_2/MeOH/H_2O = 5:1:0.1) then dissolved in water and lyophilized to afford compound **6** (26.8 mg, 73%) as white solid. R_f = 0.60 ($CHCl_3$/MeOH/H_2O = 4:1:0.1); ^{1}H NMR (400 MHz, D_2O) δ 5.13 (0.64H, d, J = 2.8 Hz), 4.67 (1.08H, m), 4.65-4.63 (0.45H, m), 4.21 (1.05H, m), 3.89-3.78 (4.74H, m), 3.74-3.56 (25.7H, m), 3.68-3.46 (1.86H, m), 3.44-3.41 (5.10H, m), 1.97 (3H, s); ^{13}C NMR (100 MHz, D_2O) δ 174.53, 174.28, 100.21, 100.18, 94.92, 90.53, 81.15, 79.72, 79.49, 74.68, 74.56, 72.37, 70.07, 69.98, 69.92, 69.63, 69.61, 69.53, 69.48, 69.21, 69.13, 68.21, 67.14, 65.78, 65.75, 60.14, 56.11, 53.71, 50.16, 50.13, 22.18, 21.88; HRMS: $[M + H]^+$ calcd for $C_{26}H_{48}N_7O_{15}^+$, 698.3203; found, 698.3229.

2-Methyl-{3,6-bis-*O*-2-[2-(2-azidoethoxy)ethoxy]ethyl-β-D-mannopyranosyl-(1→4)-1,2-dideoxy-α-D-glucopyrano}-[2,1-*d*]-2-oxazoline (7).



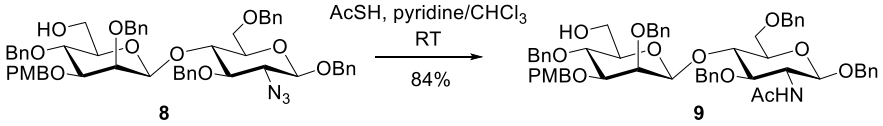
To a solution of compound **6** (5.3 mg, 7.6 μmol) in H_2O (250 μL) were added Et_3N (63.9 μL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 25.6 mg) at 0 °C. The reaction mixture was stirred at this temperature for 5 h then purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound **7** (4.8 mg, 93%) as white solid after lyophilization with 5 mol.% of NaOH. ^{1}H NMR (400 MHz, D_2O) δ 5.99 (1H, d, J = 7.2 Hz), 4.61-4.60 (1H, m), 4.28-4.26 (1H, m), 4.12-4.07 (2H, m), 3.82-3.77 (3H, m), 3.69-3.61 (24H, m), 3.57-3.55 (1H, m), 3.54-3.53 (1H, m), 3.42-3.39 (4H, m), 3.38-3.35 (1H, m), 3.33-3.29 (1H, m), 1.97 (3H, s); ^{13}C NMR (100 MHz, D_2O) δ 168.16, 100.80, 99.50, 80.75, 77.26, 74.64, 70.53, 69.82, 69.68, 69.53, 69.22, 69.15, 69.09, 68.80, 68.78, 67.75, 66.65, 65.53, 64.86, 61.27, 49.78, 49.75, 12.56; HRMS: $[M + H]^+$ calcd for $C_{26}H_{46}N_7O_{14}^+$, 680.3097; found, 680.3125.

Benzyl 2,4-di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (8)



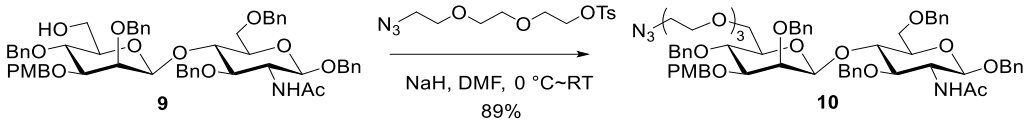
To a solution of compound **3** (300 mg, 0.321 mmol) in BH_3·THF (1 M, 4.0 mL) was added a solution of Bu_2BOTf in CH_2Cl_2 (1 M, 642 µL) under argon atmosphere at 0 °C and the mixture was stirred at 0 °C for 40 min when TLC indicated the completion of the reaction. Et_3N (300 µL) was added to the reaction followed by careful addition of MeOH (600 µL). The mixture was co-evaporated with MeOH three times and the residue was purified by flash chromatography (hexanes/EtOAc = 5:1~2:1) to afford **8** (274 mg, 91%) as a colorless syrup. R_f = 0.30 (hexanes/EtOAc = 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50-7.28 (27H, m, Ar-H), 6.93 (2H, m, Ar-H), 5.16 (1H, d, PhC*H*$_2$, *J* = 10.8 Hz), 5.01 (1H, d, PhC*H*$_2$, *J* = 12.1 Hz), 4.97-4.86 (3H, m, PhC*H*$_2$), 4.78 (1H, d, PhC*H*$_2$, *J* = 12.1 Hz), 4.75-4.64 (3H, m, PhC*H*$_2$), 4.55-4.52 (3H, m, PhC*H*$_2$), 4.40 (1H, d, *J* = 8.1 Hz), 4.01 (1H, dd, *J* = 9.3 Hz, *J* = 9.3 Hz), 3.89-3.75 (6H, m), 3.73-3.67 (2H, m), 3.57 (1H, dd, *J* = 8.2 Hz, *J* = 8.2 Hz), 3.45-3.39 (4H, m), 3.22-3.18 (1H, m), 1.97 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.31, 138.76, 138.52, 138.47, 137.75, 136.93, 130.38, 129.20, 128.64, 128.56, 128.45, 128.38, 128.26, 128.17, 128.06, 128.03, 127.91, 127.78, 127.69, 127.65, 127.51, 113.89, 100.76, 100.51, 82.34, 81.50, 77.08, 75.80, 75.31, 75.10, 74.90, 74.83, 74.57, 73.71, 71.65, 70.93, 68.54, 65.96, 62.22, 55.34; MALDI-TOF: $[M + Na]^+$ calcd for $C_{55}H_{59}N_3NaO_{11}^+$, 960.40; found, 959.98.

Benzyl 2,4-di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (9)



A solution of compound **8** (133.5 mg, 0.142 mmol) in a mixture of AcSH/pyridine/$CHCl_3$ (0.8 mL/0.6 mL/0.8 mL) was stirred at room temperature for 18 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc = 4:1~3:2) to afford compound **9** (113.8 mg, 84%) as colorless syrup. R_f = 0.30 (hexanes/EtOAc = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.43 (2H, m, Ar-H), 7.37-7.23 (25H, m, Ar-H), 6.88-6.86 (2H, m, Ar-H), 5.75 (1H, d, N*H*, *J* = 8.0 Hz), 4.98-4.81 (6H, m, PhC*H*$_2$), 4.65-4.59 (4H, m, PhC*H*$_2$), 4.53-4.47 (4H, m, PhC*H*$_2$), 4.16 (1H, dd, *J* = 7.8 Hz, *J* = 7.8 Hz), 3.90 (1H, dd, *J* = 7.0 Hz, *J* = 7.0 Hz), 3.86-3.78 (6H, m), 3.73-3.69 (3H, m), 3.64-3.58 (1H, m), 3.53-3.48 (1H, m), 3.42-3.39 (1H, m), 3.22-3.18 (1H, m), 2.09 (1H, s), 1.76 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.54, 159.25, 138.78, 138.63, 138.37, 137.91, 137.60, 130.28, 129.17, 128.51, 128.39, 128.33, 128.26, 128.15, 128.02, 127.88, 127.74, 127.66, 127.61, 127.54, 113.84, 101.08, 99.14, 82.18, 77.78, 75.71, 75.47, 75.13, 75.08, 74.76, 74.62, 73.75, 73.57, 71.59, 70.71, 69.38, 62.28, 55.29, 23.38; MALDI-TOF: $[M + Na]^+$ calcd for $C_{57}H_{63}NNaO_{12}^+$, 976.42; found, 976.00.

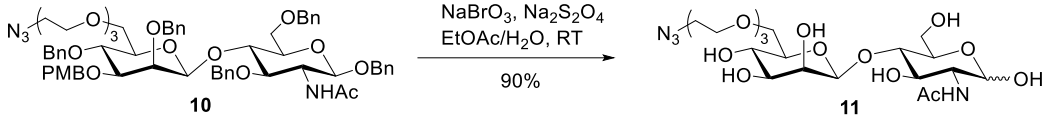
Benzyl 2,4-di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-6-*O*-2-[2-(2-azidoethoxy)ethoxy]ethyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (10).



To a solution of compound **9** (88.0 mg, 0.092 mmol) and the tosylate linker [2] (76 mg, 0.231 mmol) in anhydrous DMF (2.4 mL) was added 60% sodium hydride (18.5 mg, 0.461 mmol) at 0 °C. After

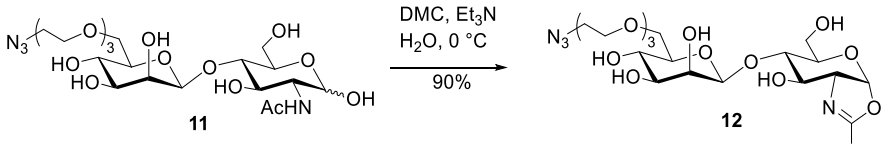
stirring for 0.5 hour at 0 °C then 6 hours at room temperature, MeOH (100 µL) and AcOH (25 µL) was added to the reaction mixture at 0 °C. The residual mixture was concentrated to dryness then purified by column chromatography on silica-gel (hexanes/Acetone = 5:1~3:2) to give compound **10** (91.4 mg, 89%) as a colorless syrup. R_f = 0.20 (hexanes/Acetone = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.43 (2H, m, Ar-H), 7.37-7.23 (25H, m, Ar-H), 6.88-6.86 (2H, m, Ar-H), 6.06 (1H, d, N*H*, *J* = 8.0 Hz), 4.94-4.82 (6H, m), 4.71-4.61 (3H, m), 4.59-4.46 (5H, m), 4.09 (1H, dd, *J* = 6.7 Hz, *J* = 6.7 Hz), 3.96 (1H, dd, *J* = 6.2 Hz, *J* = 6.2 Hz), 3.90-3.73 (9H, m), 3.68-3.52 (12H, m), 3.43 (1H, dd, *J* = 2.8 Hz, *J* = 9.4 Hz), 3.35-3.29 (3H, m), 1.71 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.14, 159.25, 139.01, 138.61, 138.58, 138.04, 137.75, 130.30, 129.22, 128.48, 128.37, 128.34, 128.24, 128.21, 128.00, 127.90, 127.83, 127.79, 127.67, 127.62, 127.36, 113.84, 101.27, 99.51, 82.17, 77.33, 77.25, 75.82, 75.47, 75.01, 74.62, 74.58, 73.50, 73.07, 71.65, 70.91, 70.61, 70.58, 70.52, 70.48, 70.40, 69.92, 69.76, 55.29, 53.42, 50.63, 23.16; MALDI-TOF: $[M + Na]^+$ calcd for $C_{63}H_{74}N_4NaO_{14}^+$, 1133.51; found, 1133.15.

6-*O*-2-[2-(2-azidoethoxy)ethoxy]ethyl-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (11).



To a solution of compound **10** (25.0 mg, 0.0225 mmol) in ethyl acetate (0.4 mL) was added a solution of $NaBrO_3$ (51.6 mg, 0.338 mmol) in water (0.25 mL). A solution of $Na_2S_2O_4$ (85 %, 60.0 mg, 0.293 mmol) in water (0.50 mL) was added over 10 min at 0 °C and the mixture was vigorously stirred for 7 h at room temperature. The mixture was quenched with saturated sodium thiosulphate and concentrated in vacuum. The residue was purified by flash chromatography (CH_2Cl_2/MeOH = 10:1~ CH_2Cl_2/MeOH/H_2O = 2:1:0.1) then dissolved in water and lyophilized to afford compound **11** (11.0 mg, 90%) as white solid. R_f = 0.30 ($CHCl_3$/MeOH/H_2O = 4:1:0.1); ^{1}H NMR (400 MHz, D_2O) δ 5.13 (0.70H, d, *J* = 2.7 Hz), 4.65-4.63 (0.47H, m), 4.01-3.99 (1.08H, m), 3.90-3.85 (1.01H, m), 3.84-3.81 (1.98H, m), 3.81-3.78 (1.06H, m), 3.77-3.76 (0.26H, m), 3.75-3.71 (1.45H, m), 3.70-3.61 (14.3H, m), 3.61-3.56 (1.34H, m), 3.55 (0.37H, m), 3.53 (0.69H, m), 3.50-3.47 (1.57H, m), 3.45-3.42 (2.29H, m), 1.97 (3H, s); ^{13}C NMR (100 MHz, D_2O) δ 174.53, 174.27, 100.26, 100.22, 94.91, 90.52, 79.70, 79.47, 74.78, 74.57, 72.74, 72.38, 70.51, 70.04, 69.99, 69.89, 69.84, 69.63, 69.61, 69.53, 69.21, 69.14, 66.68, 66.65, 60.29, 60.15, 56.12, 53.71, 50.16, 22.18, 21.88; HRMS: $[M + H]^+$ calcd for $C_{20}H_{37}N_4O_{13}^+$, 541.2352; found, 541.2369.

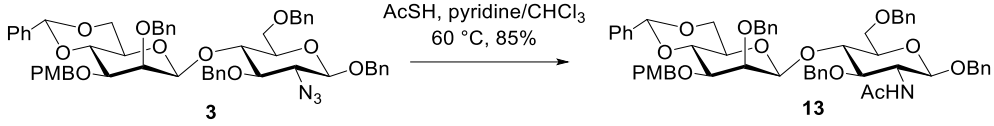
2-Methyl-{6-*O*-2-[2-(2-azidoethoxy)ethoxy]ethyl-β-D-mannopyranosyl-(1→4)-1,2-dideoxy-α-D-glucopyrano}-[2,1-*d*]-2-oxazoline (12).



To a solution of compound **11** (7.5 mg, 13.8 µmol) in H_2O (300 µL) were added Et_3N (77.3 µL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 46.9 mg) at 0 °C. The reaction mixture was stirred at this temperature for 5 h then purified by gel filtration on a Sephadex G-10 column that was

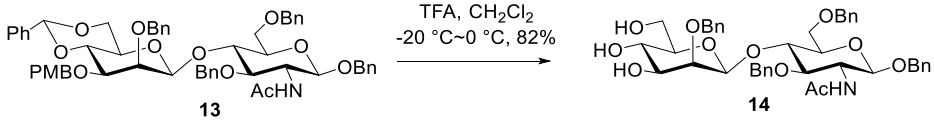
eluted with 0.1% aq Et_3N to afford compound **12** (6.5 mg, 90%) as white solid after lyophilization with 5 mol.% of NaOH. ^{1}H NMR (400 MHz, D_2O) δ 6.00 (1H, d, J = 7.3 Hz), 4.64-4.63 (1H, m), 4.29-4.27 (1H, m), 4.12-4.09 (1H, m), 3.91-3.89 (1H, m), 3.84-3.76 (2H, m), 3.70-3.67 (4H, m), 3.66-3.63 (10H, m), 3.59-3.51 (3H, m), 3.49-3.47 (1H, m), 3.44-3.41 (3H, m), 3.35-3.31 (1H, m), 1.99 (3H, d, J = 1.7 Hz); ^{13}C NMR (100 MHz, D_2O) δ 168.59, 101.23, 99.87, 77.53, 75.10, 72.73, 70.91, 70.39, 70.18, 70.06, 69.63, 69.61, 69.54, 69.45, 69.22, 66.80, 65.19, 61.68, 50.15, 12.96; HRMS: $[M + H]^+$ calcd for $C_{20}H_{35}N_4O_{12}^+$, 523.2246; found, 523.2267.

Benzyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (13)



A solution of compound **3** (200.0 mg, 0.214 mmol) in a mixture of AcSH/pyridine/$CHCl_3$ (1.0 mL/0.8 mL/1.0 mL) was stirred at 60 °C for 18 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc = 4:1~1:1) to afford compound **13** (170.0 mg, 85%) as colorless syrup. R_f = 0.15 (hexanes/EtOAc = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53-7.50 (2H, m, Ar-H), 7.44-7.25 (25H, m, Ar-H), 6.88-6.86 (2H, m, Ar-H), 5.87 (1H, d, N*H*, J = 8.0 Hz), 5.57 (1H, s, PhC*H*), 4.98-4.86 (4H, m, PhCH_2), 4.82-4.79 (1H, d, J = 11.7 Hz, PhCH_2), 4.71-4.68 (1H, d, J = 12.0 Hz, PhCH_2), 4.63-4.55 (5H, m), 4.46-4.43 (1H, d, J = 12.0 Hz, PhCH_2), 4.15-4.05 (3H, m), 3.94 (1H, dd, J = 7.1 Hz, J = 7.1 Hz), 3.84-3.76 (5H, m), 3.69-3.60 (4H, m), 3.48 (1H, dd, J = 2.9 Hz, J = 9.9 Hz), 3.20-3.14 (1H, m), 1.75 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.33, 159.21, 149.48, 138.79, 138.38, 137.90, 137.59, 130.49, 129.15, 128.89, 128.50, 128.46, 128.37, 128.22, 128.20, 128.01, 127.91, 127.89, 127.75, 127.68, 127.51, 126.11, 123.87, 113.78, 101.81, 101.42, 99.25, 78.70, 78.01, 77.83, 77.27, 77.19, 75.29, 75.18, 73.67, 73.55, 72.36, 70.75, 69.31, 68.58, 67.34, 55.29, 23.25; MALDI-TOF: $[M + Na]^+$ calcd for $C_{57}H_{61}NNaO_{12}^+$, 974.41; found, 974.07.

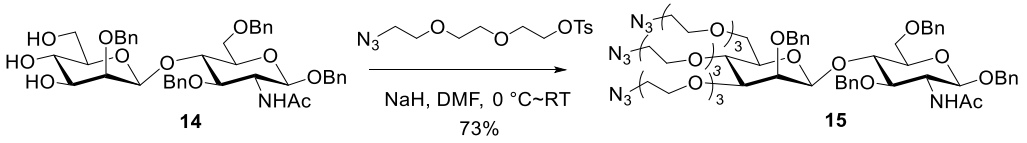
Benzyl 2-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (14)



To a solution of compound **13** (148.5 mg, 0.156 mmol) in CH_2Cl_2 (8.0 mL) was added Trifluoroacetic acid (1.5 mL) at -20 °C. After stirring at this temperature for 30 min, the reaction was warmed to 0 °C and stirred for further 1 hour. After the completion of the reaction as monitored by TLC, the reaction was quenched by MeOH (4 mL) at 0 °C and extracted by CH_2Cl_2, the residual mixture was concentrated to dryness then purified by column chromatography on silica-gel (hexanes/Acetone = 4:1~1:2) to give compound **14** (95.0 mg, 82%) as a colorless syrup. R_f = 0.20 (hexanes/Acetone = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.24 (20H, m, Ar-H), 6.09 (1H, m, N*H*), 5.00-4.89 (4H, m, PhCH_2), 4.68-4.50 (6H, m), 4.15 (1H, dd, J = 8.2 Hz, J = 8.2 Hz), 3.92 (1H, dd, J = 8.0 Hz, J = 8.0 Hz), 3.81-3.64 (4H, m), 3.60-3.59 (1H, m), 3.52-3.47 (4H, m), 3.25-3.20 (1H, m), 3.07-3.03 (1H, m), 2.84 (1H,

s), 2.35 (1H, s), 1.75 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.77, 138.49, 138.18, 137.74, 137.52, 128.58, 128.48, 128.39, 128.34, 128.07, 128.00, 127.97, 127.81, 101.16, 99.08, 78.12, 77.82, 77.73, 77.31, 76.07, 75.31, 75.04, 74.34, 73.94, 73.63, 70.86, 68.99, 68.84, 62.22, 55.85, 29.30, 23.36; MALDI-TOF: $[M + Na]^+$ calcd for $C_{42}H_{49}NNaO_{11}^+$, 766.32; found, 766.00.

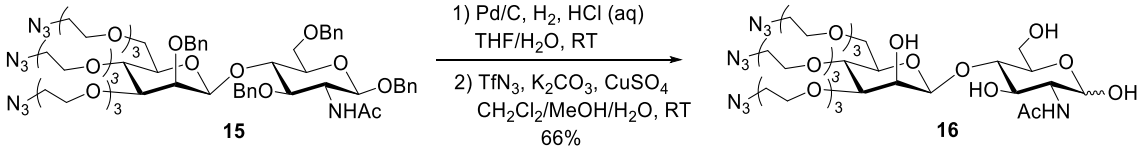
Benzyl 2-*O*-benzyl-3,4,6-tri-*O*-2-[2-(2-azidoethoxy)ethoxy]ethyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (15).



14 15

To a solution of compound **9** (84.0 mg, 0.113 mmol) and the tosylate linker [2] (205 mg, 0.623 mmol) in anhydrous DMF (3.2 mL) was added 60% sodium hydride (36.0 mg, 0.900 mmol) at 0 °C. After stirring for 0.5 hour at 0 °C then 10 hours at room temperature, MeOH (200 µL) and AcOH (40 µL) was added to the reaction mixture at 0 °C. The residual mixture was concentrated to dryness then purified by column chromatography on silica-gel (hexanes/Acetone = 4:1~1:1) to give compound **15** (99.7 mg, 73%) as a colorless syrup. R_f = 0.50 (hexanes/Acetone = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.23 (20H, m, Ar-H), 6.08 (1H, d, J = 8.4 Hz, N*H*), 4.91-4.76 (5H, m, PhCH_2), 4.67-4.55 (3H, m), 4.49-4.46 (2H, m), 4.06-3.88 (4H, m), 3.82-3.71 (6H, m), 3.69-3.53 (30H, m), 3.51-3.48 (2H, m), 3.40-3.37 (2H, m), 3.34-3.32 (4H, m), 3.29-3.22 (2H, m), 1.68 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.58, 138.50, 138.20, 137.56, 137.25, 127.94, 127.79, 127.66, 127.34, 127.30, 127.23, 127.07, 127.02, 126.80, 100.54, 99.02, 82.91, 76.50, 75.24, 75.04, 74.91, 74.64, 74.04, 72.96, 72.48, 71.51, 70.41, 70.35, 70.22, 70.19, 70.11, 70.05, 69.98, 69.94, 69.90, 69.79, 69.55, 69.50, 69.40, 69.22, 69.16, 52.69, 50.18, 50.13, 22.60; MALDI-TOF: $[M + Na]^+$ calcd for $C_{60}H_{82}N_{10}NaO_{17}^+$, 1237.58; found, 1237.46.

3,4,6-tri-*O*-2-[2-(2-azidoethoxy)ethoxy]ethyl-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (16).

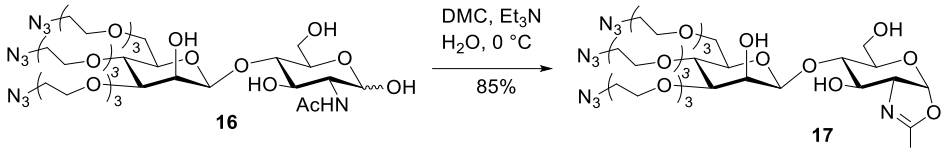


15 16

A mixture of compound **15** (99.7 mg, 0.082 mmol) and Pd/C (10 wt.% loading, 40 mg) in THF (4.5 mL) and H_2O (1.5 mL) was added 2 M HCl (aq, 205 µL, 5 eq), then stirred under H_2 atmosphere overnight. After LC-MS analysis showed the complete conversion to free amine intermediate, the reaction mixture was filtered through a Celite pad, then concentrated to dryness. To the solution of the residue in H_2O (3.0 mL) was added freshly prepared solution of TfN_3 [3] in CH_2Cl_2 (4.0 mL, 1.23 mmol), K_2CO_3 (92 mg) and $CuSO_4$ (10 mg) at 0 °C, then MeOH was added to make the solution homogenous, and the mixture was stirred at room temperature for 24 h. The reaction mixture was filtered, then concentrated to dryness and purified on a Sephadex LH-20 column by elution with H_2O. Fractions containing the product were pooled and lyophilized, then further purified by preparative RP-HPLC (gradient, 20−35% aq MeCN containing 0.1% FA for 40 min; flow rate, 10.0 mL/min) to give compound **16** (46.2 mg, 66%) as white solid. ^{1}H NMR (400 MHz, D_2O) δ 5.10 (0.56H, d, J = 3.0 Hz), 4.63 (0.84H, m), 4.62-4.59 (0.54H, m), 4.17-4.15 (1.12H, m), 3.92-3.88 (1.66H, m), 3.87-3.83 (1.76H,

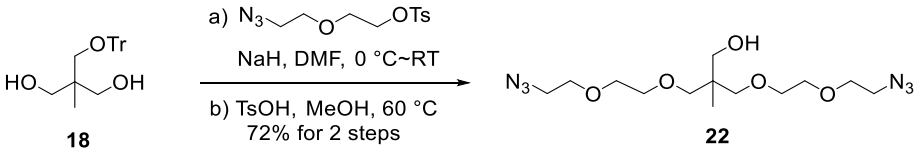
m), 3.81-3.75 (5.13H, m), 3.69-3.58 (33.19H, m), 3.50-3.45 (4.06H, m), 3.43-3.39 (6.41H, m), 3.55 (0.37H, m), 3.53 (0.69H, m), 3.50-3.47 (1.57H, m), 3.45-3.42 (2.29H, m), 1.96-1.94 (3H, m); ^{13}C NMR (100 MHz, D_2O) δ 174.07, 173.81, 99.68, 99.65, 94.47, 90.08, 81.00, 79.21, 78.97, 74.12, 74.03, 73.99, 73.33, 71.89, 71.18, 69.59, 69.56, 69.24, 69.19, 69.17, 69.13, 68.82, 68.78 ,68.66 ,68.04, 67.13, 59.83, 59.70, 55.68, 53.26, 49.73, 49.71, 21.73, 21.44; HRMS: $[M + H]^+$ calcd for $C_{32}H_{59}N_{10}O_{17}^+$, 855.4054; found, 855.4088.

2-Methyl-{3,4,6-tri-*O*-2-[2-(2-azidoethoxy)ethoxy]ethyl-β-D-mannopyranosyl-(1→4)-1,2-dideoxy-α-D-glucopyrano}-[2,1-*d*]-2-oxazoline (17).



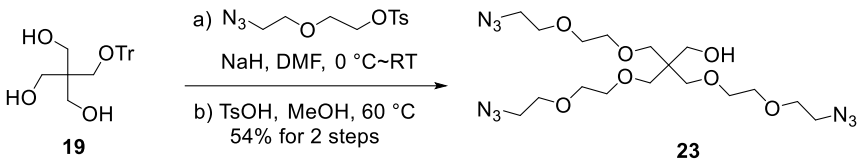
To a solution of compound **16** (3.0 mg, 3.5 μmol) in H_2O (250 μL) were added Et_3N (29.5 μL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 11.9 mg) at 0 °C. The reaction mixture was stirred at this temperature for 8 h then purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound **17** (2.5 mg, 85%) as colorless oil after lyophilization with 5 mol.% of NaOH. ^{1}H NMR (400 MHz, D_2O) δ 6.01 (1H, d, J = 7.3 Hz), 4.61-4.60 (1H, m), 4.30-4.29 (1H, m), 4.13-4.10 (2H, m), 3.96-3.91 (2H, m), 3.83-3.77 (3H, m), 3.69-3.61 (35H, m), 3.46-3.41 (11H, m), 3.36-3.31 (1H, m), 1.99 (3H, d, J = 1.7 Hz); ^{13}C NMR (100 MHz, D_2O) δ 168.16, 100.75, 99.49, 81.06, 77.26, 74.23, 73.72, 71.20, 70.52, 69.78, 69.65, 69.63, 69.33, 69.29, 69.23, 69.20, 69.16, 69.03, 68.86, 68.83, 68.03, 67.08, 64.85, 61.28, 46.13, 12.56; HRMS: $[M + H]^+$ calcd for $C_{32}H_{57}N_{10}O_{16}^+$, 837.3949; found, 837.3981.

Synthesis of scaffold (22).



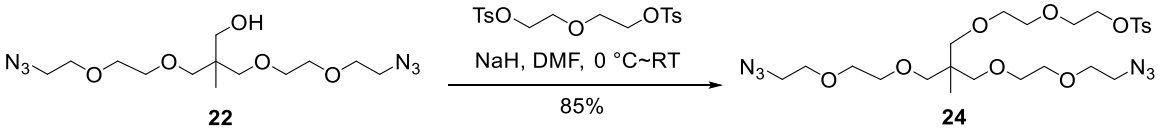
To a solution of compound **18** [4] (400 mg, 1.10 mmol) and the tosylate linker [5] (944 mg, 3.30 mmol) in anhydrous DMF (5.0 mL) was added 60% sodium hydride (198 mg, 4.95 mmol) at 0 °C. After stirring overnight at room temperature, MeOH (200 μL) was added at 0 °C to quench the reaction. The residual mixture was diluted with CH_2Cl_2, washed with saturated NH_4Cl (aq.) and brine, dried over Na_2SO_4 and concentrated to obtain the crude product. To the solution of the crude product in MeOH (10 mL) was added $TsOH·H_2O$ (62.7 mg, 0.33 mmol), and the reaction was heated to 60 °C and stirred for 2 h. After the completion of the reaction as monitored by TLC, the reaction was concentrated to dryness then purified by column chromatography on silica-gel (hexanes/EtOAc = 4:1~1:1) to give compound **22** (272 mg, 72%) as a colorless syrup. R_f = 0.20 (hexanes/EtOAc = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69-3.60 (12H, m), 3.58-3.55 (2H, m), 3.51-3.49 (2H, m), 3.45-3.43 (2H, m), 3.40-3.37 (4H, m), 3.04-3.01 (1H, m), 0.88 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 75.66, 71.00, 70.46, 70.05, 68.90, 50.76, 40.70, 17.45; ESI-TOF (positive mode): $[M + Na]^+$ calcd for $C_{13}H_{26}N_6NaO_5^+$, 369.19; found, 369.06.

Synthesis of scaffold (23).



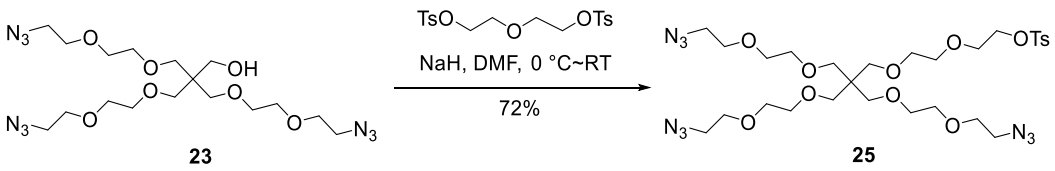
To a solution of compound **19** [6] (400 mg, 1.06 mmol) and the tosylate linker [5] (1.21 g, 4.24 mmol) in anhydrous DMF (5.0 mL) was added 60% sodium hydride (212 mg, 5.30 mmol) at 0 °C. After stirring overnight at room temperature, MeOH (250 µL) was added at 0 °C to quench the reaction. The residual mixture was diluted with CH_2Cl_2, washed with saturated NH_4Cl (aq.) and brine, dried over Na_2SO_4 and concentrated to obtain the crude product. To the solution of the crude product in MeOH (10 mL) was added $TsOH \cdot H_2O$ (61.0 mg, 0.32 mmol), and the reaction was heated to 60 °C and stirred for 2 h. After the completion of the reaction as monitored by TLC, the reaction was concentrated to dryness then purified by column chromatography on silica-gel (hexanes/EtOAc = 4:1~1:2) to give compound **23** (270 mg, 54%) as a colorless syrup. R_f = 0.20 (hexanes/EtOAc = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69-3.61 (20H, m), 3.54-3.52 (6H, m), 3.40-3.38 (6H, m), 3.06-3.03 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 71.62, 71.01, 70.41, 70.03, 65.59, 50.77, 45.10; MALDI-TOF: $[M + Na]^+$ calcd for $C_{17}H_{33}N_9NaO_7^+$, 498.24; found, 498.10.

Synthesis of scaffold (24).



To a solution of compound **22** (294 mg, 0.850 mmol) and the tosylate linker [7] (1.76 g, 4.25 mmol) in anhydrous DMF (10.0 mL) was added 60% sodium hydride (170 mg, 4.25 mmol) at 0 °C. After stirring overnight at room temperature, MeOH (200 µL) was added at 0 °C to quench the reaction. The residual mixture was diluted with CH_2Cl_2, washed with saturated NH_4Cl (aq.) and brine, dried over Na_2SO_4 and concentrated, then purified by column chromatography on silica-gel (CH_2Cl_2, then hexanes/Acetone = 2:1~1:1) to give compound **24** (426 mg, 85%) as a colorless syrup. R_f = 0.50 (hexanes/Acetone = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.80 (2H, m, Ar-H), 7.37-7.34 (2H, m, Ar-H), 4.17-4.15 (2H, m), 3.72-3.67 (6H, m), 3.65-3.63 (4H, m), 3.59-3.55 (6H, m), 3.52-3.50 (2H, m), 3.39-3.37 (4H, m), 3.33-3.30 (6H, m), 2.46 (3H, s), 0.94 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.79, 133.05, 129.83, 127.97, 73.89, 73.84, 71.09, 71.03, 70.58, 70.55, 70.03, 69.34, 68.69, 50.81, 40.99, 21.64, 17.34; MALDI-TOF: $[M + Na]^+$ calcd for $C_{24}H_{40}N_6NaO_9S^+$, 611.25; found, 611.04.

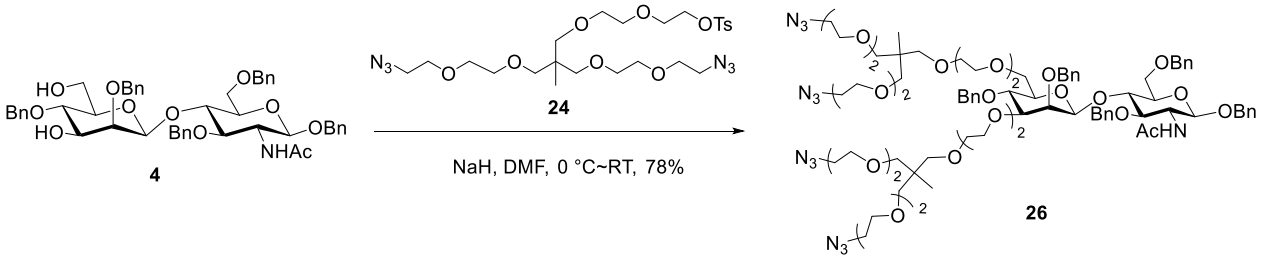
Synthesis of scaffold (25).



To a solution of compound **23** (250 mg, 0.526 mmol) and the tosylate linker [7] (1.09 g, 2.63 mmol) in anhydrous DMF (7.0 mL) was added 60% sodium hydride (105 mg, 2.63 mmol) at 0 °C. After stirring overnight at room temperature, MeOH (200 µL) was added at 0 °C to quench the reaction. The residual mixture was diluted with CH_2Cl_2, washed with saturated NH_4Cl (aq.) and brine, dried over Na_2SO_4

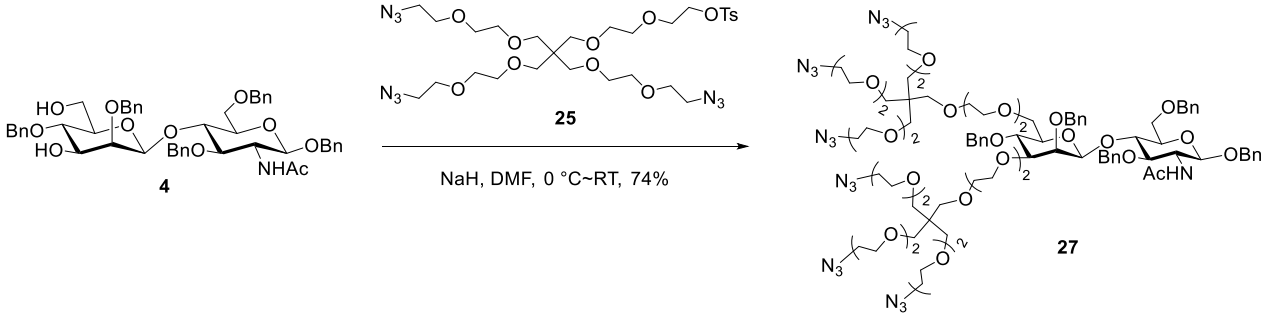
and concentrated, then purified by column chromatography on silica-gel (CH_2Cl_2, then hexanes/Acetone = 4:1~1:1) to give compound **25** (272 mg, 72%) as a colorless syrup. R_f = 0.40 (hexanes/EtOAc = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.80 (2H, m, Ar-H), 7.37-7.35 (2H, m, Ar-H), 4.16-4.14 (2H, m), 3.71-3.66 (9H, m), 3.64-3.62 (7H, m), 3.59-3.55 (8H, m), 3.52-3.50 (2H, m), 3.48-3.42 (8H, m), 3.39-3.36 (6H, m), 2.46 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.80, 133.05, 129.83, 127.97, 71.03, 70.98, 70.50, 69.99, 69.34, 68.66, 50.80, 45.54, 21.63; MALDI-TOF: [M + Na]$^+$ calcd for $C_{28}H_{47}N_9NaO_{11}S^+$, 740.30; found, 740.02.

Synthesis of compound (26).



To a solution of compound **4** (40.0 mg, 0.048 mmol) and scaffold **24** (113 mg, 0.192 mmol) in anhydrous DMF (1.5 mL) was added 60% sodium hydride (15.4 mg, 0.384 mmol) at 0 °C. After stirring for 0.5 hour at 0 °C then 11 hours at room temperature, MeOH (200 μL) was added at 0 °C to quench the reaction. The residual mixture was diluted with CH_2Cl_2, washed with brine, dried over Na_2SO_4 and concentrated, then purified by column chromatography on silica-gel (hexanes/Acetone = 4:1~3:2) to give compound **26** (62.4 mg, 78%) as a colorless syrup. R_f = 0.30 (hexanes/Acetone = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.24 (25H, m, Ar-H), 6.11 (1H, d, J = 8.3 Hz, N*H*), 4.92-4.78 (6H, m, PhC*H*$_2$), 4.69-4.45 (6H, m), 4.07 (1H, dd, J = 6.5 Hz, J = 6.5 Hz), 3.95-3.87 (2H, m), 3.83-3.80 (2H, m), 3.80-3.72 (3H, m), 3.70-3.47 (42H, m), 3.40-3.26 (22H, m), 1.69 (3H, s), 0.95 (3H, s), 0.94 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.11, 138.97, 138.69, 138.64, 138.04, 137.75, 128.46, 128.34, 128.31, 128.27, 128.18, 128.01, 127.89, 127.84, 127.76, 127.61, 127.58, 127.56, 127.33, 101.09, 99.51, 83.71, 77.29, 75.72, 75.56, 75.18, 74.84, 74.54, 74.44, 73.95, 73.91, 73.86, 73.47, 73.00, 71.09, 70.91, 70.80, 70.55, 70.53, 70.47, 70.24, 70.04, 70.03, 69.75, 69.65, 53.19, 50.79, 41.01, 41.00, 23.11, 17.35; MALDI-TOF: [M + Na]$^+$ calcd for $C_{83}H_{119}N_{13}NaO_{23}^+$, 1688.84; found, 1688.35.

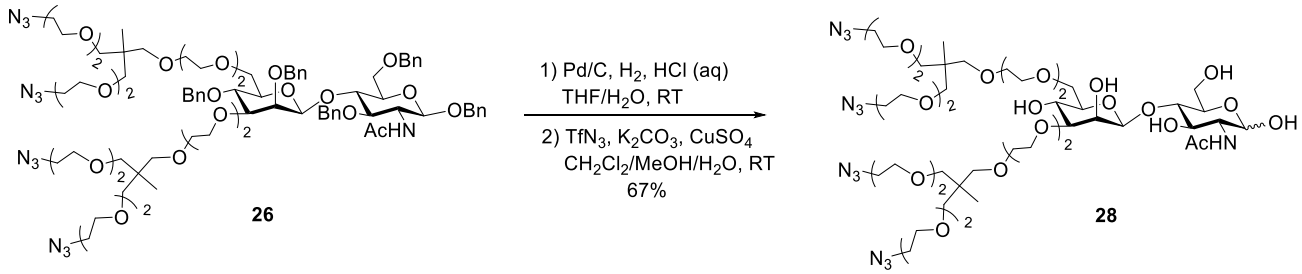
Synthesis of compound (27).



To a solution of compound **4** (35.0 mg, 0.042 mmol) and scaffold **25** (122 mg, 0.170 mmol) in anhydrous DMF (1.3 mL) was added 60% sodium hydride (13.4 mg, 0.335 mmol) at 0 °C. After stirring for 0.5 hour at 0 °C then 11 hours at room temperature, MeOH (200 μL) was added at 0 °C to quench the reaction. The residual mixture was diluted with CH_2Cl_2, washed with brine, dried over

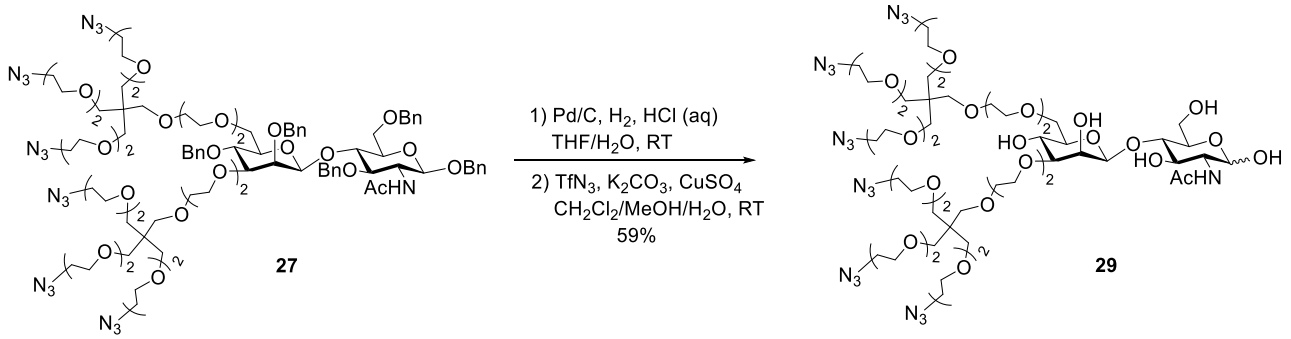
Na_2SO_4 and concentrated, then purified by column chromatography on silica-gel (hexanes/Acetone = 6:1~3:2) to give compound **27** (60.0 mg, 74%) as a colorless syrup. R_f = 0.30 (hexanes/Acetone = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.41 (2H, m, Ar-H), 7.37-7.24 (23H, m, Ar-H), 6.12 (1H, d, *J* = 8.4 Hz, N*H*), 4.92-4.78 (6H, m, PhCH_2), 4.67-4.45 (6H, m), 4.07 (1H, dd, *J* = 6.6 Hz, *J* = 6.6 Hz), 3.93 (1H, dd, *J* = 6.1 Hz, *J* = 6.1 Hz), 3.88-3.85 (1H, m), 3.84-3.78 (2H, m), 3.78-3.70 (4H, m), 3.68-3.64 (13H, m), 3.64-3.59 (18H, m), 3.57-3.51 (18H, m), 3.50-3.43 (20H, m), 3.42 (2H, m), 3.38-3.33 (12H, m), 3.32-3.26 (2H, m), 1.69 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.12, 138.98, 138.68, 138.64, 138.04, 137.74, 128.46, 128.34, 128.31, 128.26, 128.17, 128.00, 127.94, 127.90, 127.84, 127.76, 127.62, 127.59, 127.56, 127.33, 101.06, 99.49, 83.70, 75.72, 75.52, 75.17, 74.82, 74.54, 74.44, 73.47, 73.05, 71.03, 70.98, 70.87, 70.85, 70.73, 70.49, 70.47, 70.41, 70.19, 70.06, 70.04, 69.99, 69.98, 69.71, 69.60, 53.34, 50.79, 45.55, 45.53, 23.11; MALDI-TOF: $[M + Na]^+$ calcd for $C_{91}H_{133}N_{19}NaO_{27}^+$, 1946.95; found, 1947.43.

Synthesis of compound (28).



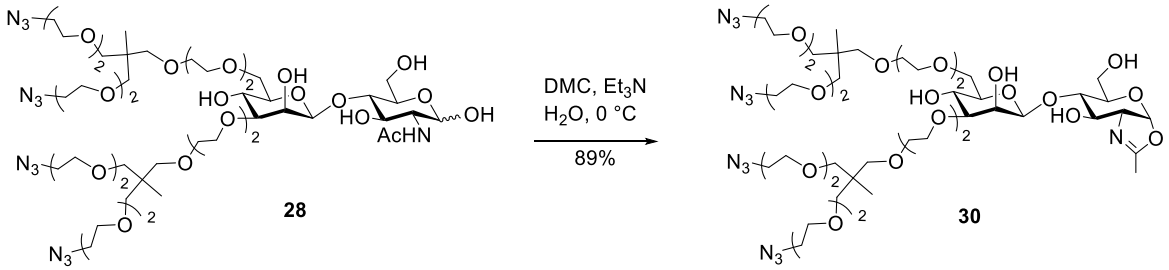
A mixture of compound **26** (70.0 mg, 0.042 mmol) and Pd/C (10 wt.% loading, 35 mg) in THF (3.0 mL) and H_2O (1.0 mL) was added 2 M HCl (aq, 168 µL, 8 eq), then stirred under H_2 atmosphere overnight. After LC-MS analysis showed the complete conversion to free amine intermediate, the reaction mixture was filtered through a Celite pad, then concentrated to dryness. To the solution of the residue in H_2O (2.0 mL) was added freshly prepared solution of TfN_3 in CH_2Cl_2 (2.0 mL, 1.68 mmol), K_2CO_3 (62 mg) and $CuSO_4$ (7.2 mg) at 0 °C, then MeOH was added to make the solution homogenous, and the mixture was stirred at room temperature for 48 h. The reaction mixture was filtered, then concentrated to dryness and purified on a Sephadex LH-20 column by elution with H_2O. Fractions containing the product were pooled and lyophilized, then further purified by preparative RP-HPLC (gradient, 40−80% aq MeCN containing 0.1% FA for 40 min; flow rate, 10.0 mL/min) to give compound **28** (34.1 mg, 67%) as thick oil. ^{1}H NMR (400 MHz, D_2O) δ 5.13 (0.75H, d, *J* = 2.8 Hz), 4.68-4.65 (1.06H, m), 4.22-4.19 (1.07H, m), 3.89-3.75 (6.04H, m), 3.74-3.56 (47.07H, m), 3.52-3.34 (24.04H, m), 1.97 (3H, s), 0.88 (6H, m); ^{13}C NMR (100 MHz, D_2O) δ 174.22, 100.21, 94.92, 90.52, 81.21, 79.74, 79.52, 74.71, 74.57, 73.53, 72.37, 70.51, 70.15, 69.98, 69.94, 69.58, 69.30, 69.13, 68.22, 67.15, 65.78, 60.16, 56.12, 53.72, 50.26, 40.46, 22.21, 21.92, 16.84, 16.83; HRMS: $[M + H]^+$ calcd for $C_{48}H_{90}N_{13}O_{23}^+$, 1216.6267; found, 1216.6316.

Synthesis of compound (29).



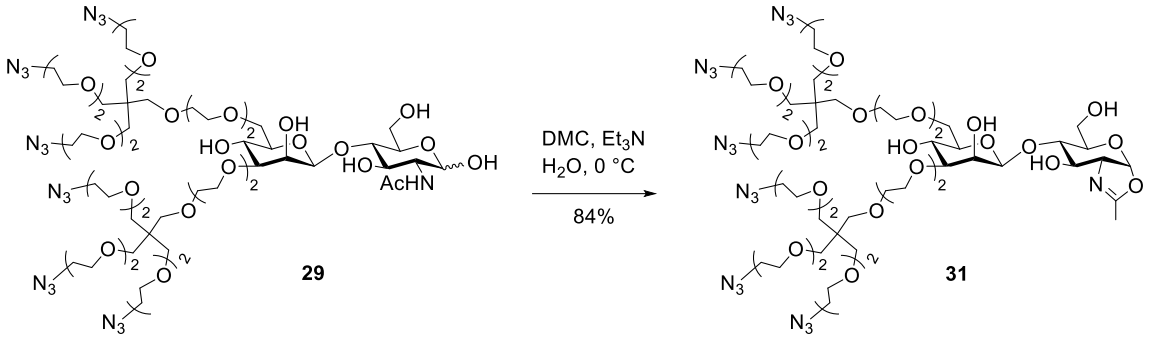
A mixture of compound **27** (50.0 mg, 0.026 mmol) and Pd/C (10 wt.% loading, 20 mg) in THF (3.0 mL) and H_2O (1.0 mL) was added 2 M HCl (aq, 105 µL, 10 eq), then stirred under H_2 atmosphere overnight. After LC-MS analysis showed the complete conversion to free amine intermediate, the reaction mixture was filtered through a Celite pad, then concentrated to dryness. To the solution of the residue in H_2O (2.0 mL) was added freshly prepared solution of TfN_3 in CH_2Cl_2 (3.0 mL, 1.56 mmol), K_2CO_3 (60 mg) and $CuSO_4$ (10 mg) at 0 °C, then MeOH was added to make the solution homogenous, and the mixture was stirred at room temperature for 48 h. The reaction mixture was filtered, then concentrated to dryness and purified on a Sephadex LH-20 column by elution with H_2O. Fractions containing the product were pooled and lyophilized, then further purified by preparative RP-HPLC (gradient, 40−80% aq MeCN containing 0.1% FA for 40 min; flow rate, 10.0 mL/min) to give compound **29** (22.5 mg, 59%) as thick oil. ^{1}H NMR (400 MHz, D_2O) δ 5.12 (0.78H, d, J = 2.8 Hz), 4.67 (1.06H, m), 4.21-4.18 (1.10H, m), 3.89-3.75 (8.11H, m), 3.74-3.56 (59.91H, m), 3.52-3.38 (30.63H, m), 1.98-1.96 (3H, m); ^{13}C NMR (100 MHz, D_2O) δ 174.48, 174.22, 100.22, 94.92, 90.52, 81.23, 79.75, 79.52, 74.70, 74.57, 72.36, 70.84, 70.55, 70.16, 69.96, 69.74, 69.64, 69.33, 69.13, 68.21, 67.13, 65.77, 60.28, 60.16, 56.12, 53.72, 50.27, 45.08, 43.38, 22.21, 21.91; HRMS: $[M + H]^+$ calcd for $C_{56}H_{104}N_{19}O_{27}^+$, 1474.7344; found, 1474.7427.

Synthesis of compound (30).



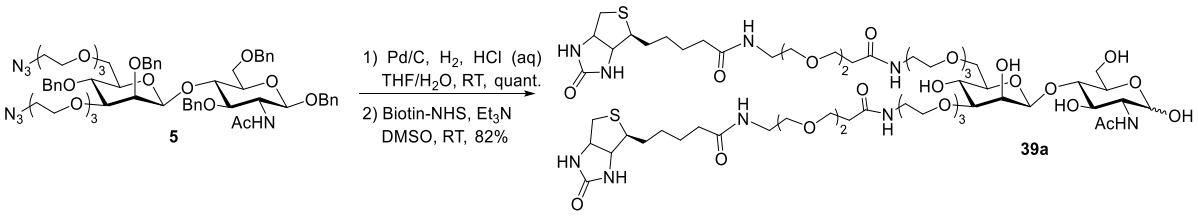
To a solution of compound **28** (9.0 mg, 7.4 µmol) in H_2O (500 µL) were added Et_3N (62.2 µL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 37.5 mg) at 0 °C. The reaction mixture was stirred at this temperature overnight then purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound **30** (7.9 mg, 89%) as colorless oil after lyophilization with 5 mol.% of NaOH. ^{1}H NMR (400 MHz, D_2O) δ 6.00 (1H, d, J = 7.3 Hz), 4.61-4.60 (1H, m), 4.28-4.27 (1H, m), 4.12-4.10 (2H, m), 3.83-3.77 (4H, m), 3.66-3.61 (34H, m), 3.69-3.61 (35H, m), 3.60-3.57 (15H, m), 3.43-3.39 (11H, m), 3.36-3.34 (14H, m), 1.99 (3H, s), 0.87 (6H, m); ^{13}C NMR (100 MHz, D_2O) δ 168.11, 100.84, 99.50, 80.80, 77.34, 74.64, 73.14, 70.52, 70.17, 70.11, 69.88, 69.74, 69.54, 69.19, 69.10, 68.87, 67.76, 66.63, 65.53, 64.89, 61.27, 46.06, 40.06, 16.41, 12.57; HRMS: $[M + H]^+$ calcd for $C_{48}H_{88}N_{13}O_{22}^+$, 1198.6161; found, 1198.6216.

Synthesis of compound (31).



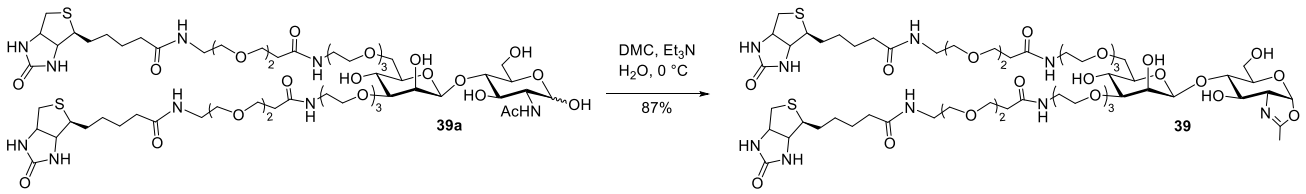
To a solution of compound **29** (10.0 mg, 6.8 μmol) in H_2O (500 μL) were added Et_3N (56.9 μL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 34.4 mg) at 0 °C. The reaction mixture was stirred at this temperature overnight then purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound **31** (8.3 mg, 84%) as colorless oil after lyophilization with 5 mol.% of NaOH. ^{1}H NMR (400 MHz, D_2O) δ 6.01 (1H, d, J = 7.3 Hz), 4.62-4.61 (1H, m), 4.30-4.29 (1H, m), 4.13-4.10 (2H, m), 3.84-3.77 (5H, m), 3.68-3.62 (38H, m), 3.61-3.57 (18H, m), 3.48-3.34 (33H, m), 2.93-2.90 (1H, m), 3.36-3.34 (14H, m), 1.99 (3H, d, J = 1.7 Hz); ^{13}C NMR (100 MHz, D_2O) δ 101.30, 99.89, 81.21, 77.78, 75.03, 70.84, 70.30, 70.16, 69.95, 69.46, 68.20, 68.12, 66.96, 65.91, 65.25, 61.68, 12.99 (selected peaks); HRMS: $[M + H]^+$ calcd for $C_{56}H_{102}N_{19}O_{26}^+$, 1456.7238; found, 1456.7310.

Synthesis of Biotin-tagged disaccharide (39a).



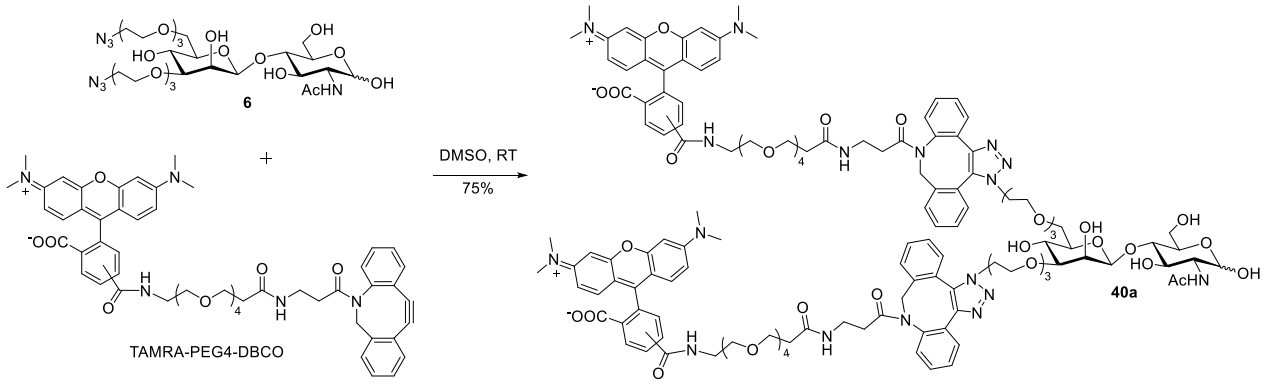
To a solution of compound **5** (56 mg, 0.049 mmol) and Pd/C (10 wt.% loading, 28 mg) in THF (3.0 mL) and H_2O (1.0 mL) was added 2 M HCl (aq, 84 μL, 3.5 eq), then stirred under H_2 atmosphere overnight. After LC-MS analysis showed the complete conversion to free amine intermediate, the reaction mixture was filtered through a Celite pad and purified by Sephadex LH-20 (H_2O) to give the intermediate in hydrochloride form (35 mg, quant.). Then a solution of the intermediate (6.6 mg, 9.2 μmol) and Biotin-NHS (11.5 mg, 0.023 mmol) in DMSO (200 μL) was added Et_3N (5.2 μL) and the mixture was incubated at room temperature until ESI-MS indicated the complete consumption of the free amine. Preparative RP-HPLC (gradient, 10−40% aq MeCN containing 0.1% FA for 40 min; flow rate, 10.0 mL/min) afforded compound **39a** (13.0 mg, 82%) as white solid. HRMS: $[M + H]^+$ calcd for $C_{60}H_{106}N_9O_{25}S_2^+$, 1416.6736; found, 1416.6799.

Synthesis of Biotin-tagged disaccharide oxazoline (39).



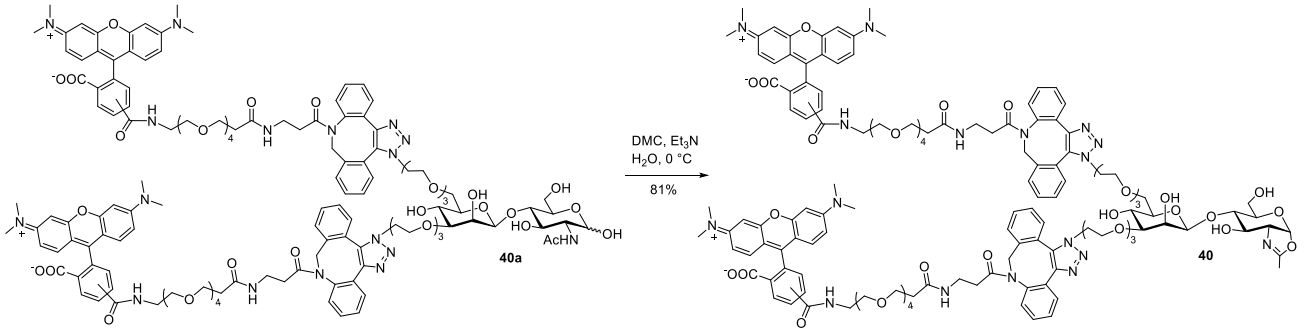
To a solution of compound **39a** (4.6 mg, 3.2 μmol) in H_2O (300 μL) were added Et_3N (18 μL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 11 mg) at 0 °C. The reaction mixture was stirred at this temperature for 8 h then purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound **39** (4.0 mg, 87%) as white solid after lyophilization with 5 mol.% of NaOH. HRMS: $[M + Na]^+$ calcd for $C_{60}H_{103}N_9NaO_{24}S_2^+$, 1420.6450; found, 1420.6499.

Synthesis of TAMRA-tagged disaccharide (40a).



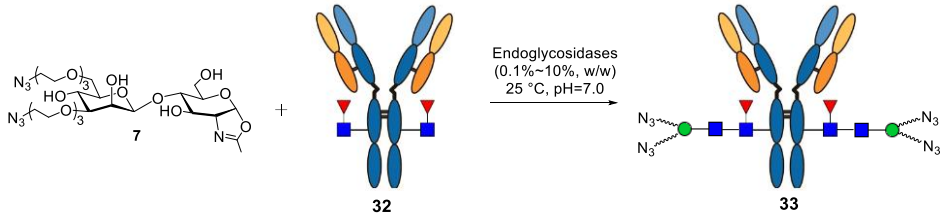
A solution of compound **6** (0.75 mg, 1.1 μmol) and TAMRA-PEG4-DBCO (3.0 mg, 3.2 μmol) in DMSO (0.6 mL) was incubated at room temperature overnight. The product was purified by semi-preparative RP-HPLC (gradient, 20−45% aq MeCN containing 0.1% FA for 40 min; flow rate, 4.0 mL/min) to give compound **40a** (2.0 mg, 75%) as purple solid. HRMS: $[M + 3H]^{3+}$ calcd for $C_{134}H_{164}N_{17}O_{35}^{3+}$, 857.3864; found, 857.3898.

Synthesis of TAMRA-tagged disaccharide oxazoline (40).



To a solution of compound **40a** (1.6 mg, 0.6 μmol) in H_2O (100 μL) were added Et_3N (5.6 μL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 3.4 mg) at 0 °C. The reaction mixture was stirred at this temperature for 8 h then purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound **40** (1.3 mg, 81%) as purple solid after lyophilization with 5 mol.% of NaOH. HRMS: $[M + 3H]^{3+}$ calcd for $C_{134}H_{162}N_{17}O_{34}^{3+}$, 851.3829; found, 851.3869.

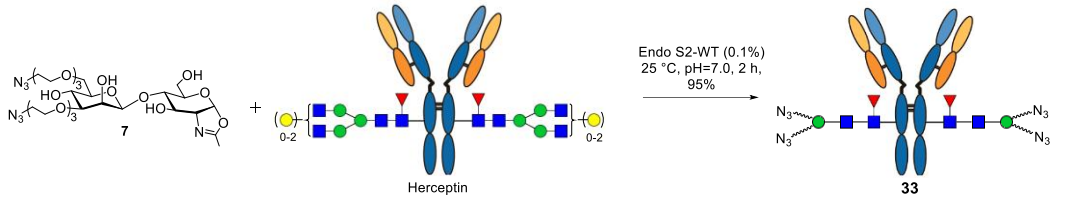
2. Screening of the transglycosylation conditions.

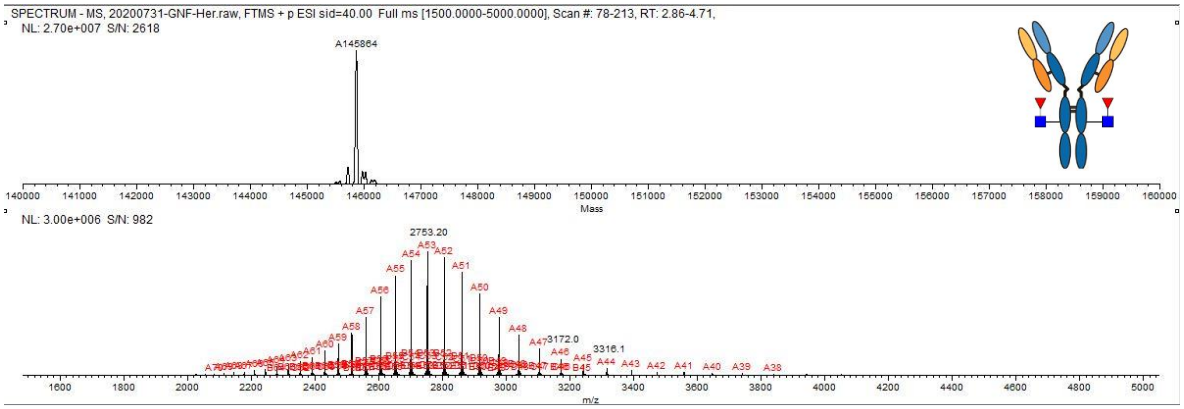


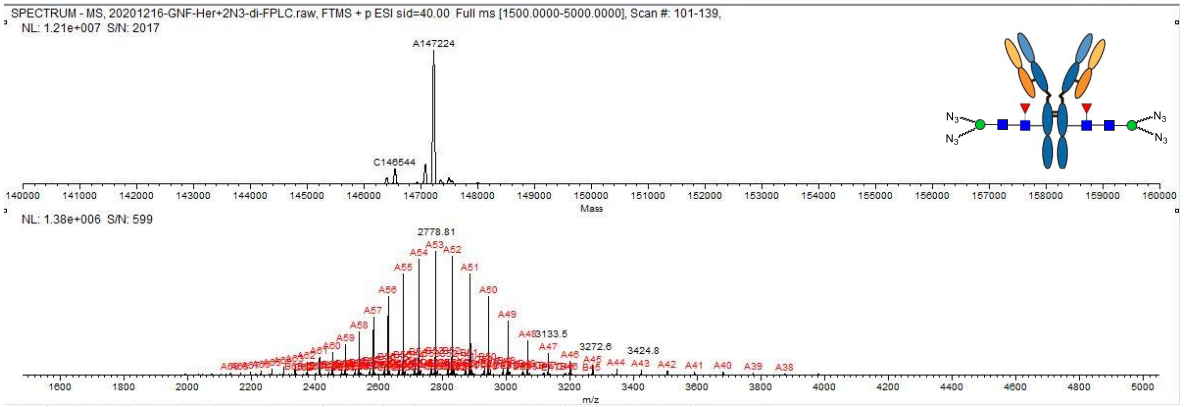
A solution of GNF-Herceptin **32** (100 μg) and oxazoline **7** (18.6 μg, 20 eq per reaction site) was incubated with different endoglycosidases (0.1 μg ~ 10 μg) at 25 °C in 5 μL of 150 mM PBS buffer (pH 7.0), and the reaction was monitored by LC-MS of aliquots. The ratio of the starting material and the product species were determined by the peak intensities, and the conversion yields were based on the reaction sites.

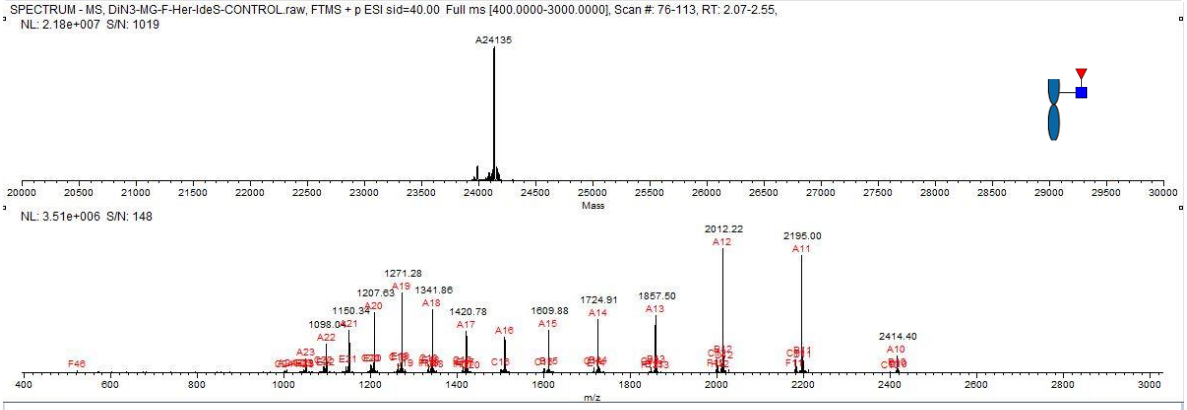
Enzymes	**Sources**
Endo-S	*Streptococcus pyogenes*
Endo-S D233Q	*Streptococcus pyogenes*
Endo-S2	*Streptococcus pyogenes M49*
Endo-S2 D184M	*Streptococcus pyogenes M49*
Endo-F3	*Elizabethkingia miricola*
Endo-F3 DA65A	*Elizabethkingia miricola*
Endo-A	*Arthrobacter protophormiae*
Endo-CC	*Coprinopsis cinerea*
Endo-D	*Streptococcus pneumoniae*

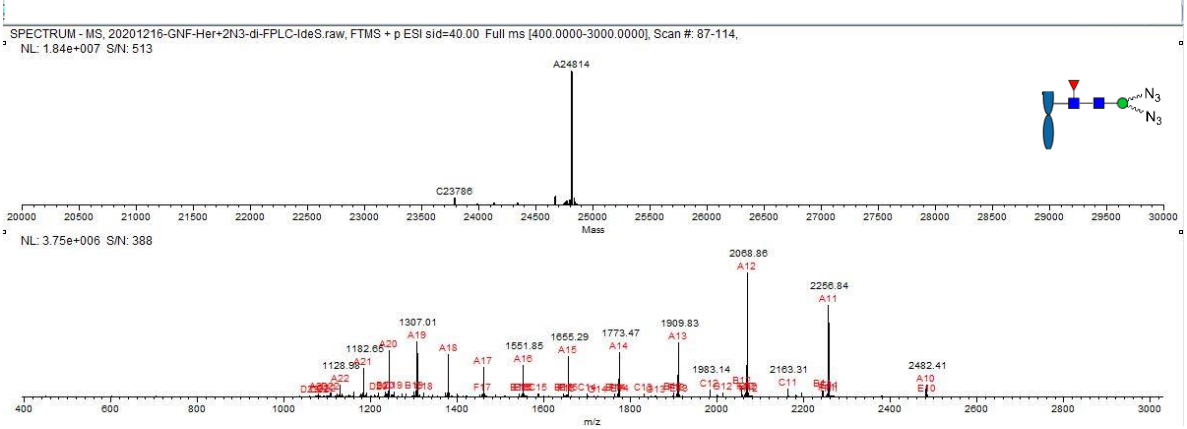
3. LC-MS analysis of the transglycosylation reactions.

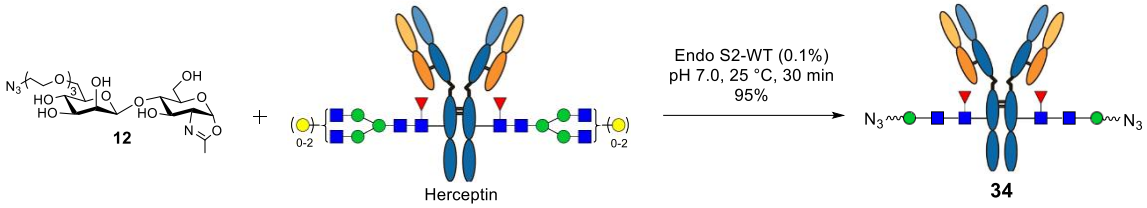


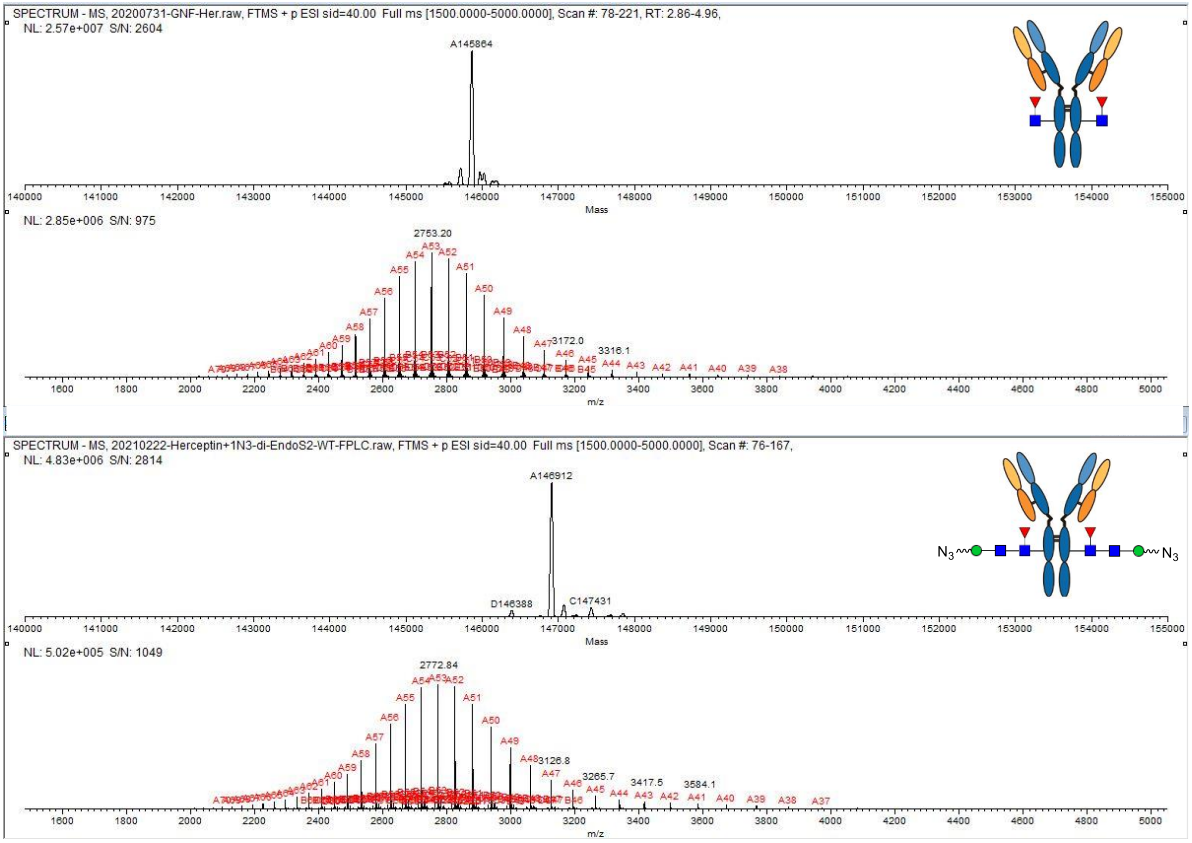









12
Herceptin
0-2
0-2
Endo S2-WT (0.1%)
pH 7.0, 25 °C, 30 min
95%
34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NL: 2.57e+007 S/N: 2604
A146864
Mass
NL: 2.85e+006 S/N: 975
2753.20
3172.0
3316.1
m/z
SPECTRUM - MS, 20210222-Herceptin+1N3-di-EndoS2-WT-FPLC.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000], Scan #: 76-167,
NL: 4.83e+006 S/N: 2814
A146912
D146388
C147431
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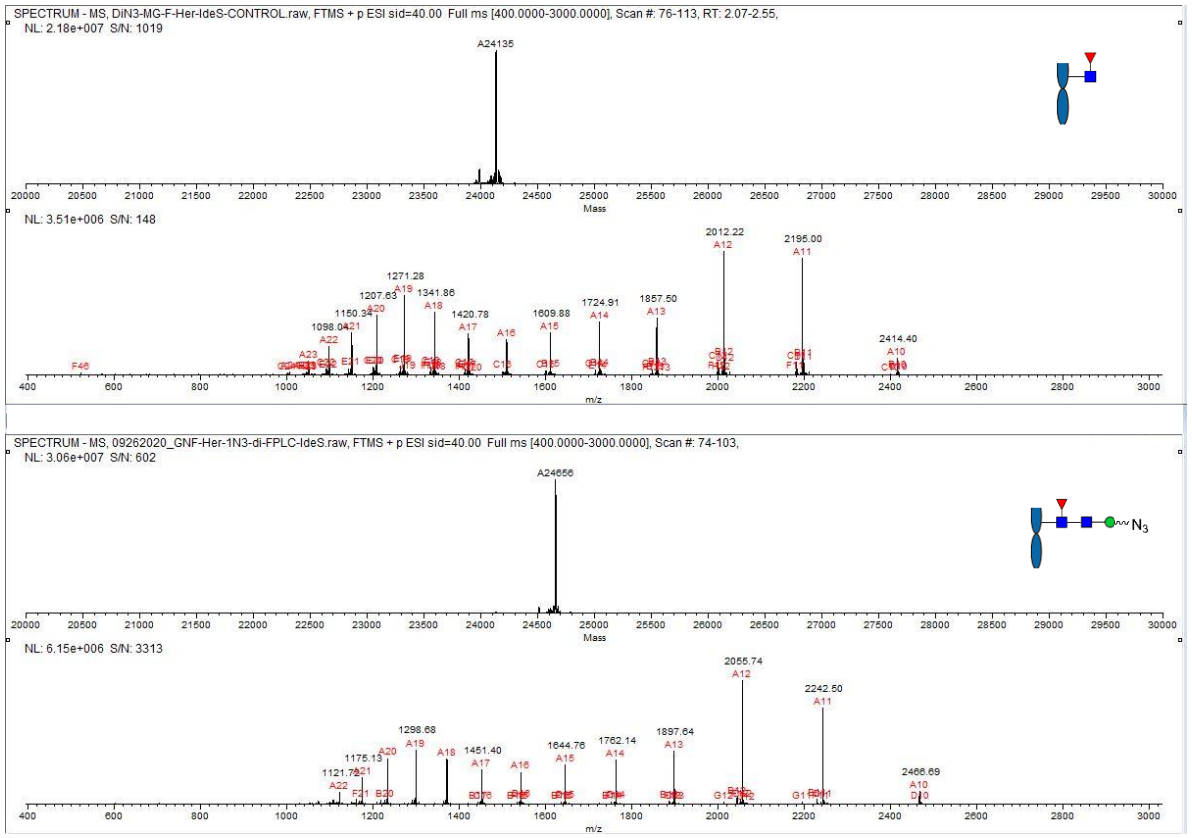
3265.7
3417.5
3584.1
m/z


SPECTRUM - MS, DiN3-MG-F-Her-IdeS-CONTROL.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 76-113, RT: 2.07-2.55,
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A24135
Mass
NL: 3.51e+006 S/N: 148
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2195.00
1271.28
1207.63
1341.86
1150.34
1420.78
1098.04
1609.88
1724.91
1857.50
2414.40
m/z
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A24656
Mass
NL: 6.15e+006 S/N: 3313
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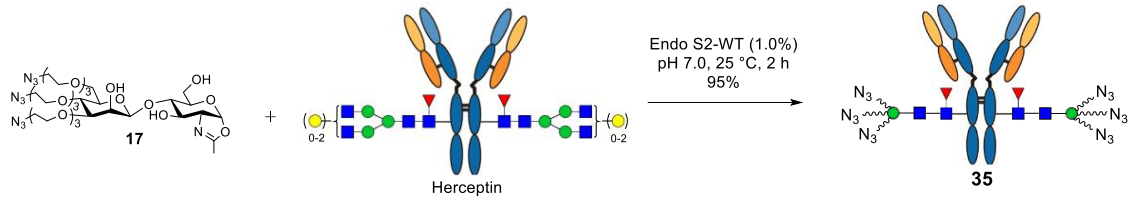
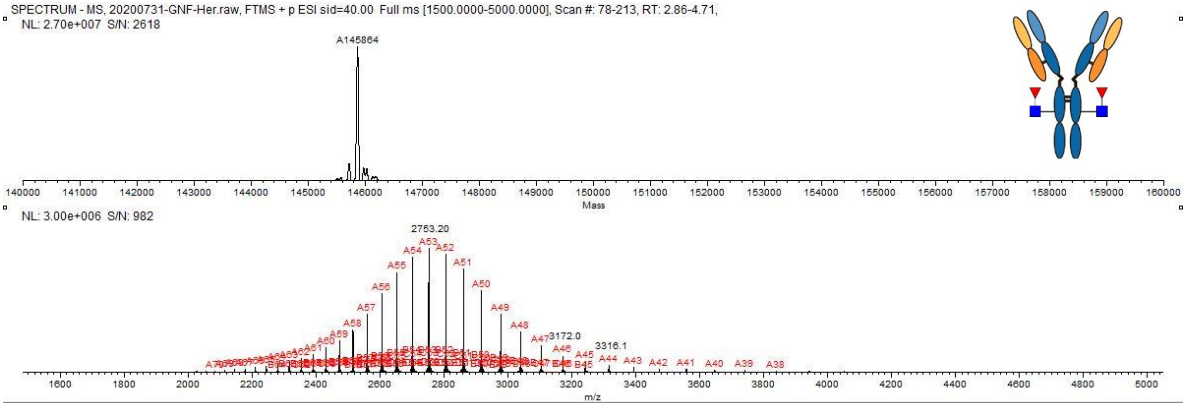
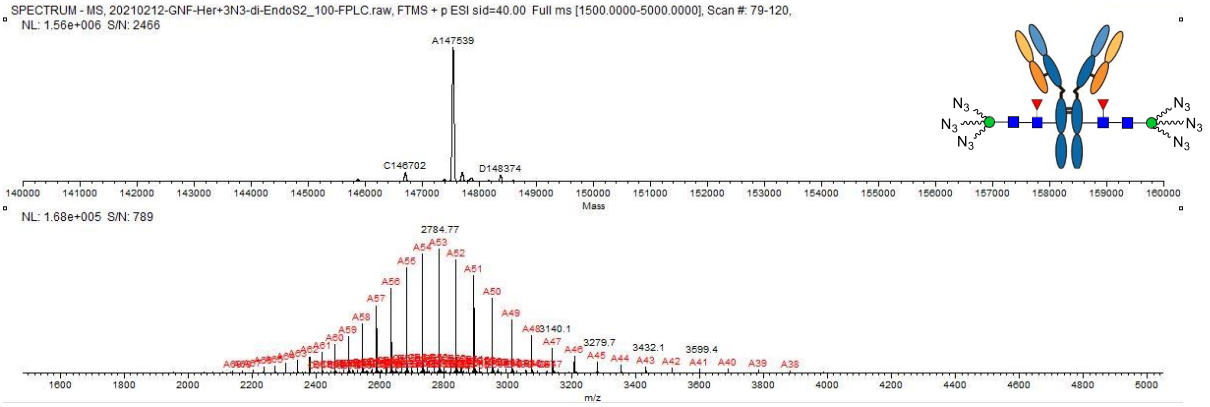
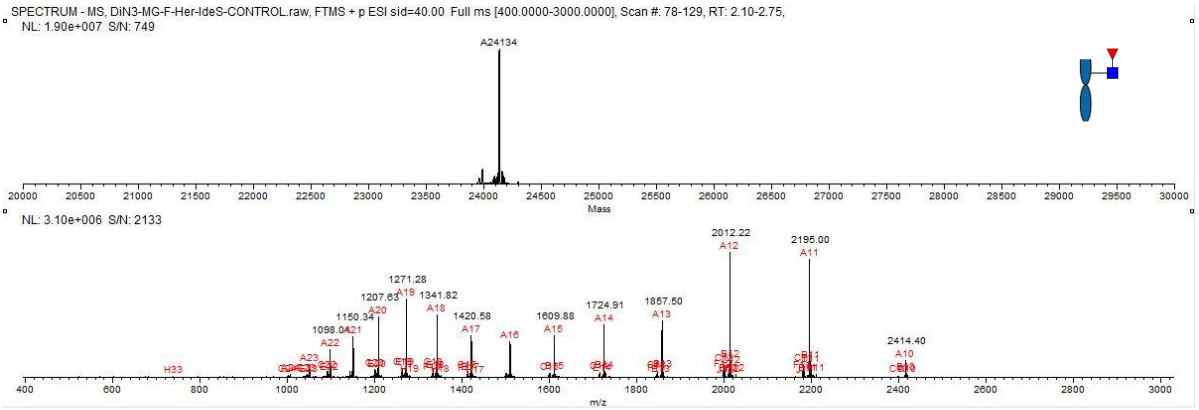
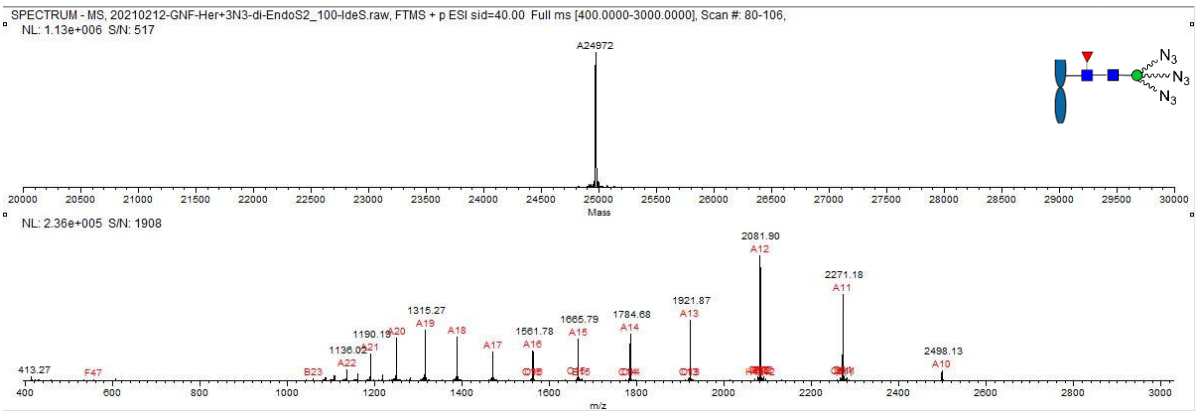
1644.76
1762.14
1897.64
2466.69
m/z

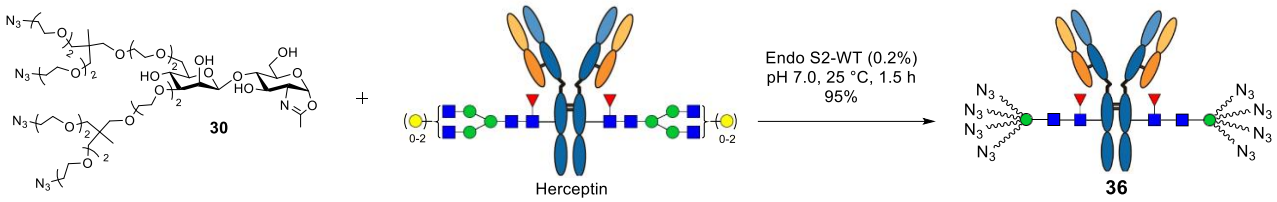

17
Herceptin
Endo S2-WT (1.0%)
pH 7.0, 25 °C, 2 h
95%
35

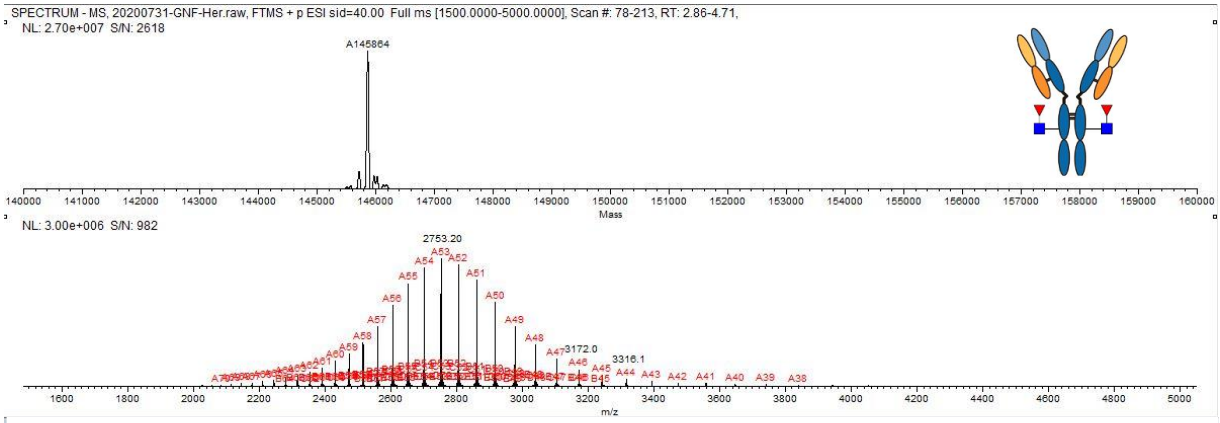

SPECTRUM - MS, 20200731-GNF-Her.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000], Scan #: 78-213, RT: 2.86-4.71,
NL: 2.70e+007 S/N: 2618
A145864
Mass
NL: 3.00e+006 S/N: 982
2753.20
3172.0
3316.1
m/z

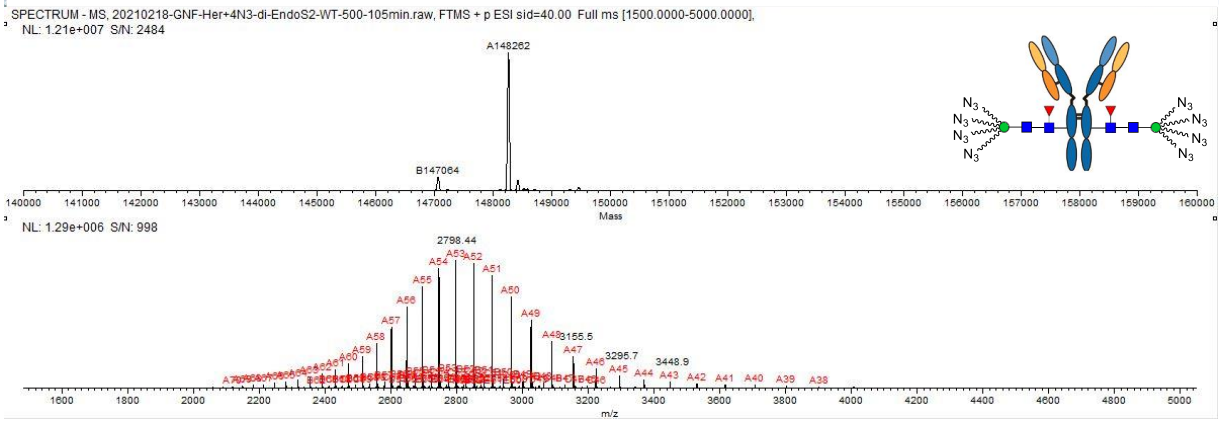

SPECTRUM - MS, 20210212-GNF-Her+3N3-di-EndoS2_100-FPLC.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000], Scan #: 79-120,
NL: 1.56e+006 S/N: 2466
A147539
C146702
D148374
Mass
NL: 1.68e+005 S/N: 789
2784.77
3140.1
3279.7
3432.1
3599.4
m/z

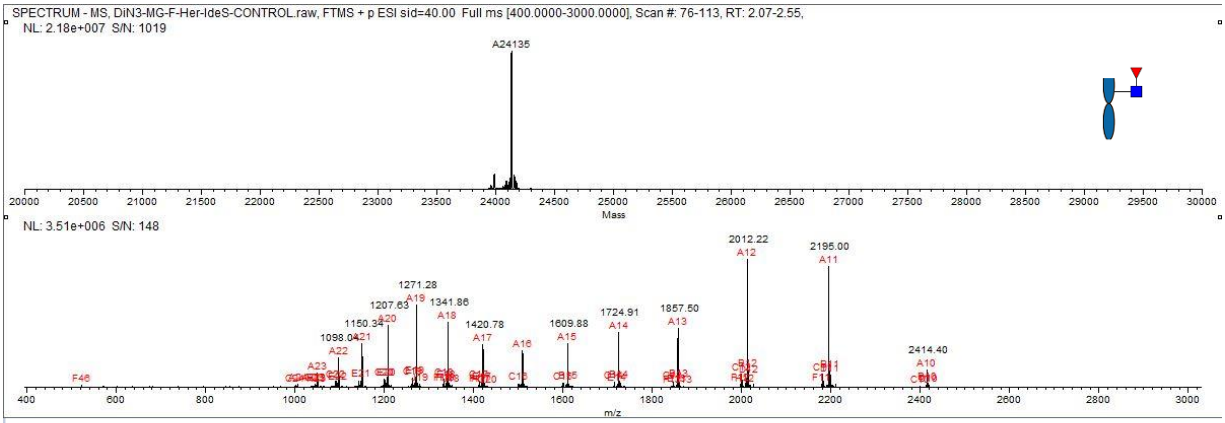

SPECTRUM - MS, DiN3-MG-F-Her-IdeS-CONTROL.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 78-129, RT: 2.10-2.75,
NL: 1.90e+007 S/N: 749
A24134
Mass
NL: 3.10e+006 S/N: 2133
2012.22
2195.00
1271.28
1341.82
1207.63
1150.34
1420.58
1098.04
1609.88
1724.91
1857.50
2414.40
m/z

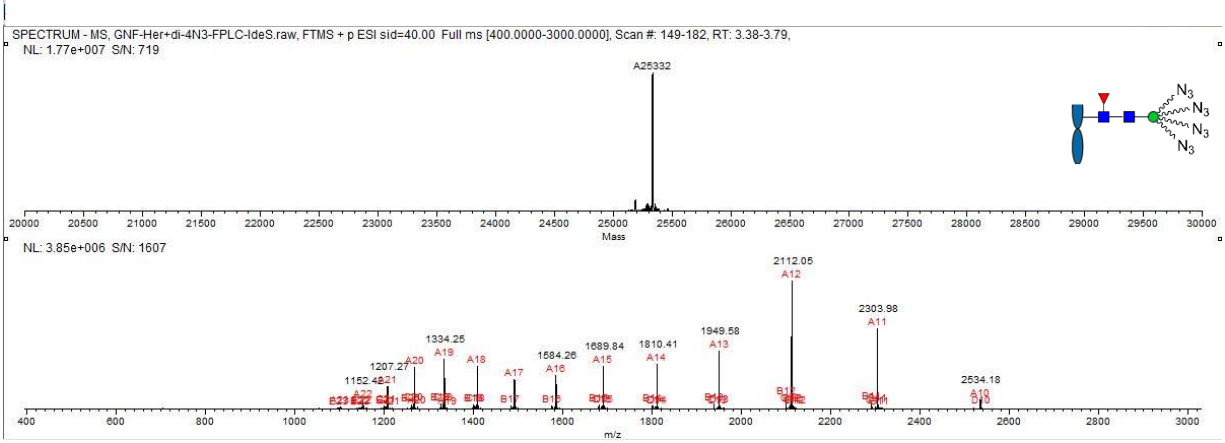

SPECTRUM - MS, 20210212-GNF-Her+3N3-di-EndoS2_100-IdeS.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 80-106,
NL: 1.13e+006 S/N: 517
A24972
Mass
NL: 2.36e+005 S/N: 1908
2081.90
2271.18
1315.27
1921.87
1784.68
1665.79
1561.78
1190.19
1136.02
2498.13
413.27
m/z

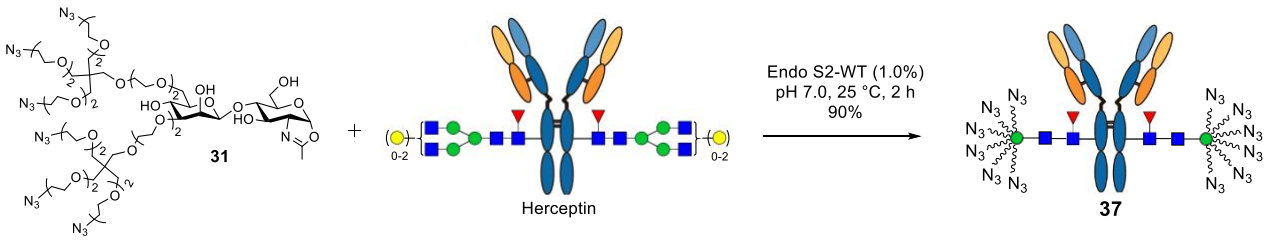

30
Herceptin
Endo S2-WT (0.2%)
pH 7.0, 25 °C, 1.5 h
95%
36

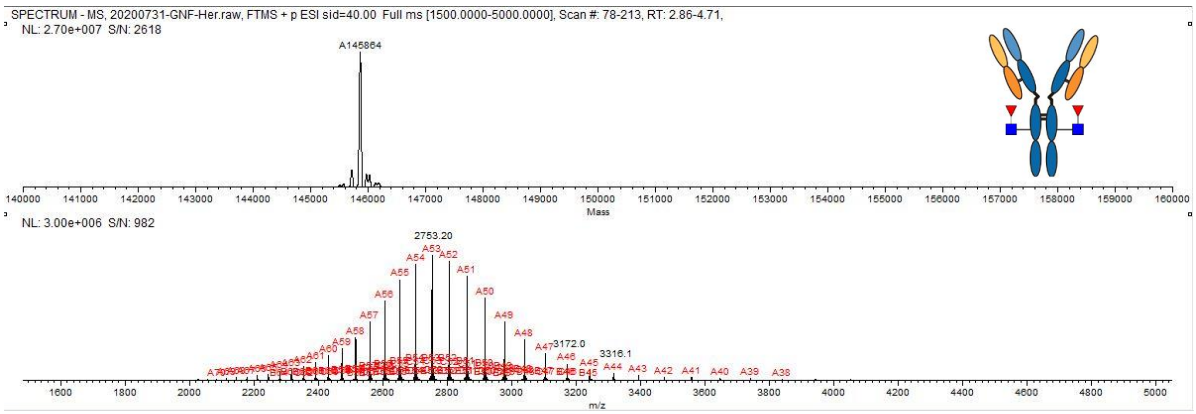

SPECTRUM - MS, 20200731-GNF-Her.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000], Scan #: 78-213, RT: 2.86-4.71,
NL: 2.70e+007 S/N: 2618
A145864
Mass
NL: 3.00e+006 S/N: 982
2753.20
3172.0
3316.1
m/z

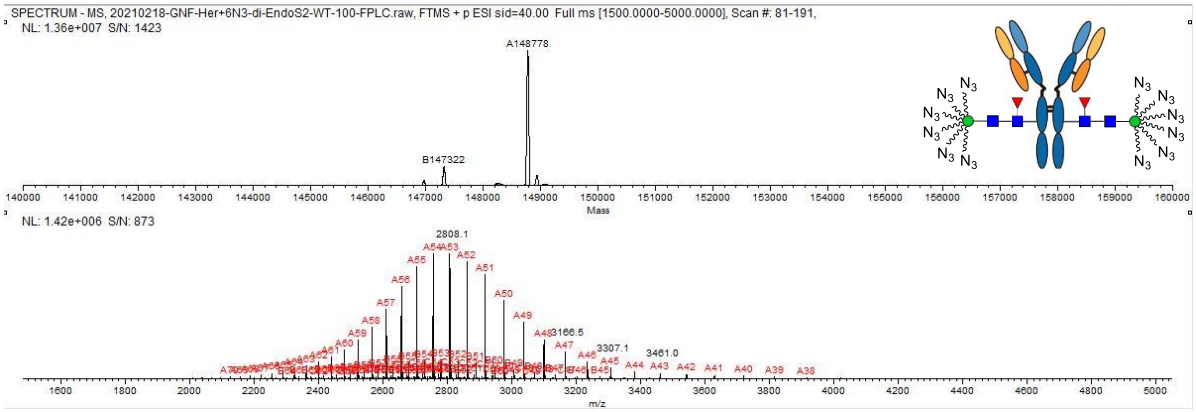

SPECTRUM - MS, 20210218-GNF-Her+4N3-di-EndoS2-WT-500-105min.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000],
NL: 1.21e+007 S/N: 2484
A148262
B147064
Mass
NL: 1.29e+006 S/N: 998
2798.44
3155.6
3295.7
3448.9
m/z

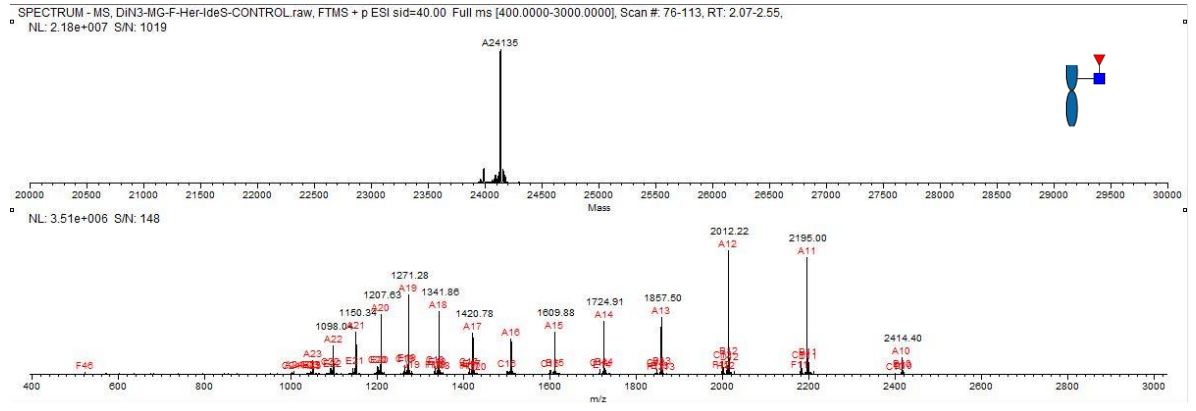

SPECTRUM - MS, DiN3-MG-F-Her-IdeS-CONTROL.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 76-113, RT: 2.07-2.55,
NL: 2.18e+007 S/N: 1019
A24135
Mass
NL: 3.51e+006 S/N: 148
2012.22
2195.00
1271.28
1207.63
1341.86
1150.34
1420.78
1098.04
1609.88
1724.91
1857.50
2414.40
m/z

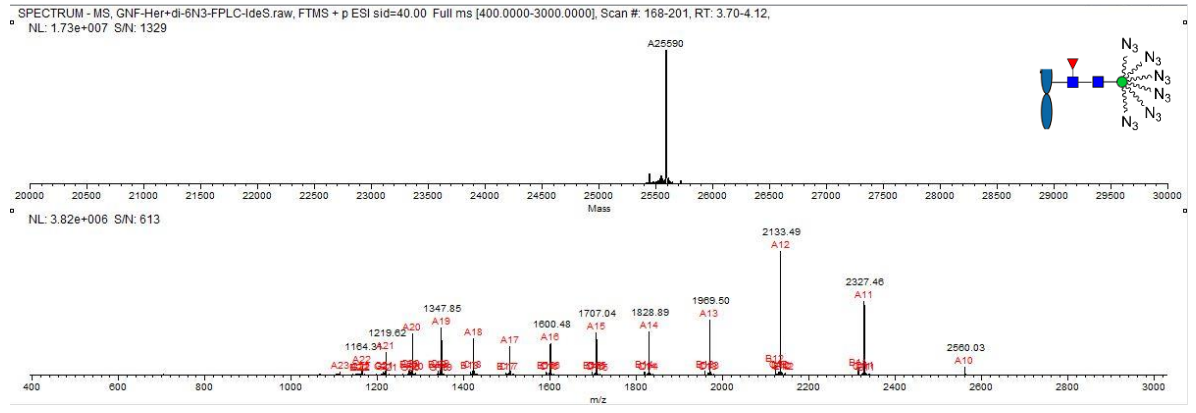

SPECTRUM - MS, GNF-Her+di-4N3-FPLC-IdeS.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 149-182, RT: 3.38-3.79,
NL: 1.77e+007 S/N: 719
A25332
Mass
NL: 3.85e+006 S/N: 1607
2112.05
2303.98
1334.25
1207.27
1152.42
1584.26
1689.84
1810.41
1949.58
2534.18
m/z

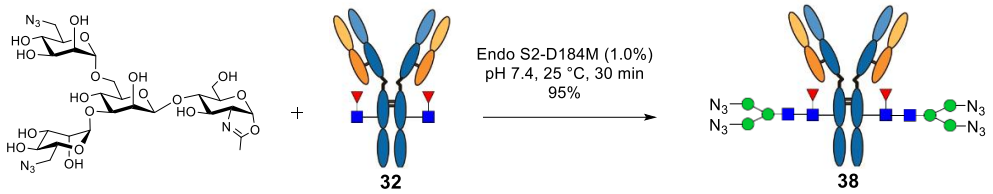

31
Herceptin
Endo S2-WT (1.0%)
pH 7.0, 25 °C, 2 h
90%
37

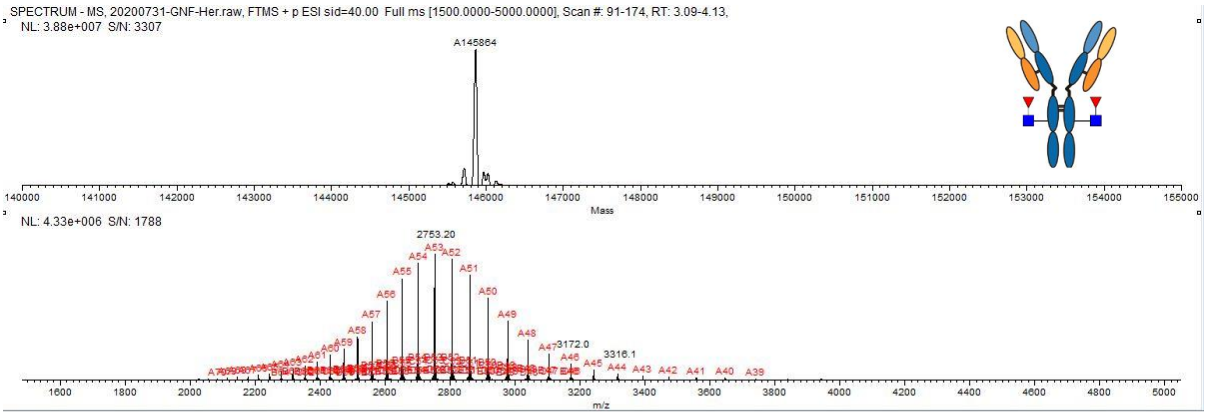

SPECTRUM - MS, 20200731-GNF-Her.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000], Scan #: 78-213, RT: 2.86-4.71,
NL: 2.70e+007 S/N: 2618
A145864
Mass
NL: 3.00e+006 S/N: 982
2753.20
3172.0
3316.1
m/z

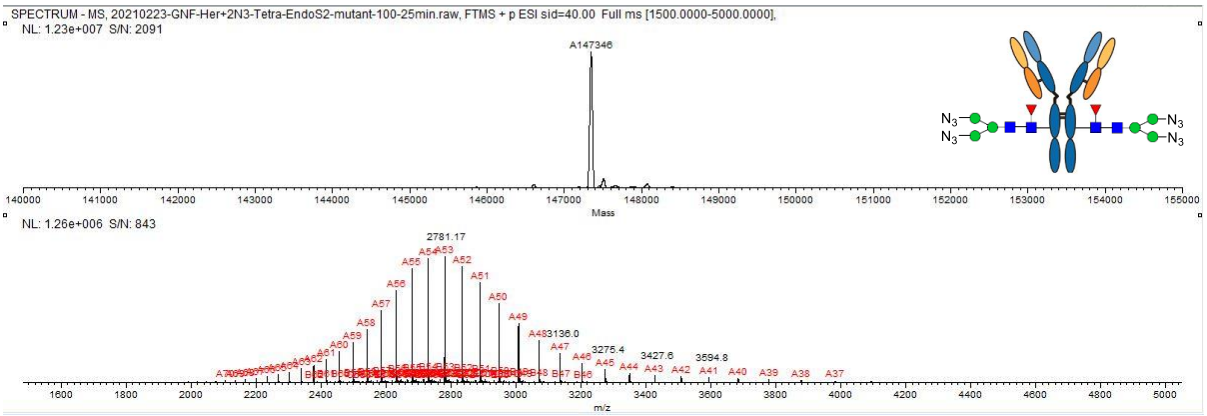

SPECTRUM - MS, 20210218-GNF-Her+6N3-di-EndoS2-WT-100-FPLC.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000], Scan #: 81-191,
NL: 1.36e+007 S/N: 1423
A148778
B147322
Mass
NL: 1.42e+006 S/N: 873
2808.1
3166.5
3307.1
3461.0
m/z

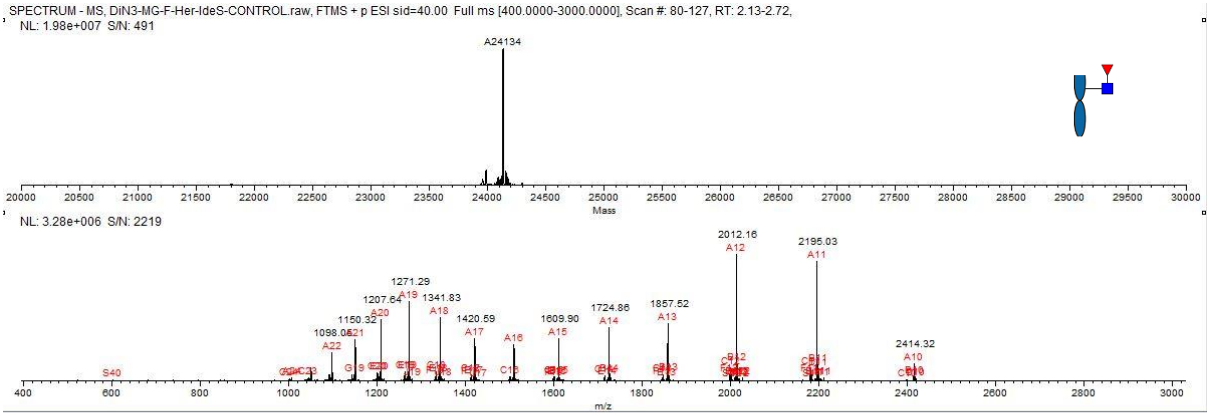

SPECTRUM - MS, DiN3-MG-F-Her-IdeS-CONTROL.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 76-113, RT: 2.07-2.55,
NL: 2.18e+007 S/N: 1019
A24135
Mass
NL: 3.51e+006 S/N: 148
2012.22
2195.00
1271.28
1207.63
1341.86
1150.34
1420.78
1098.04
1609.88
1724.91
1857.50
2414.40
m/z

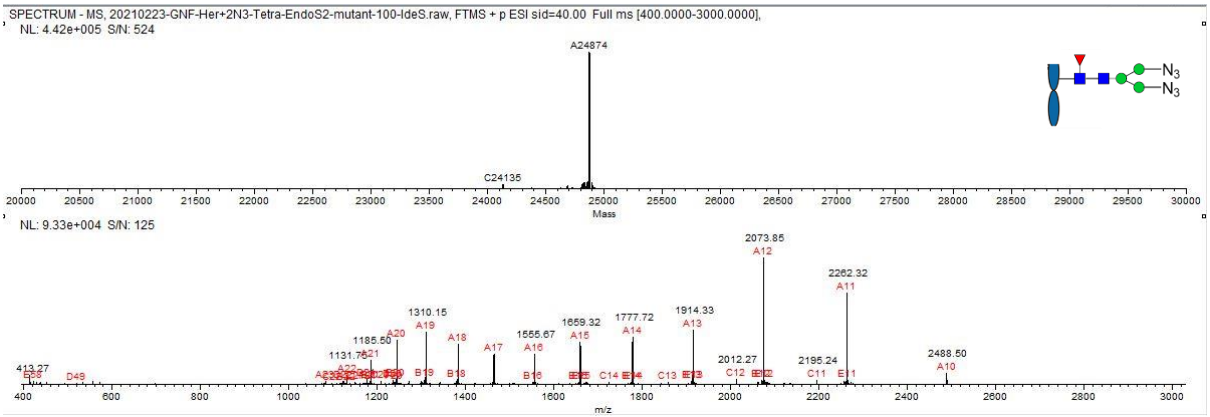

SPECTRUM - MS, GNF-Her+di-6N3-FPLC-IdeS.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 168-201, RT: 3.70-4.12,
NL: 1.73e+007 S/N: 1329
A25590
Mass
NL: 3.82e+006 S/N: 613
2133.49
2327.46
1347.85
1219.62
1164.34
1600.48
1707.04
1828.89
1969.50
2560.03
m/z

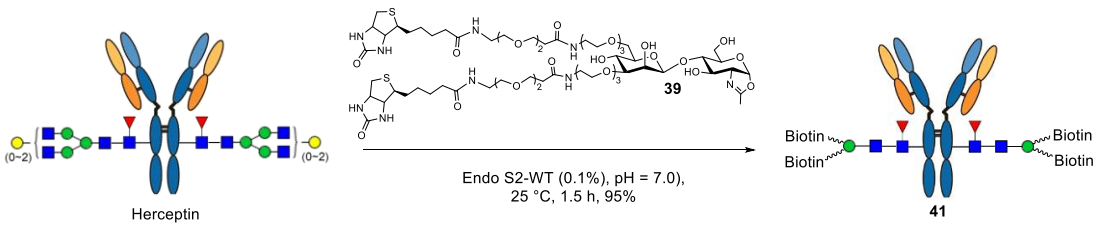

Endo S2-D184M (1.0%)
pH 7.4, 25 °C, 30 min
95%
32
38

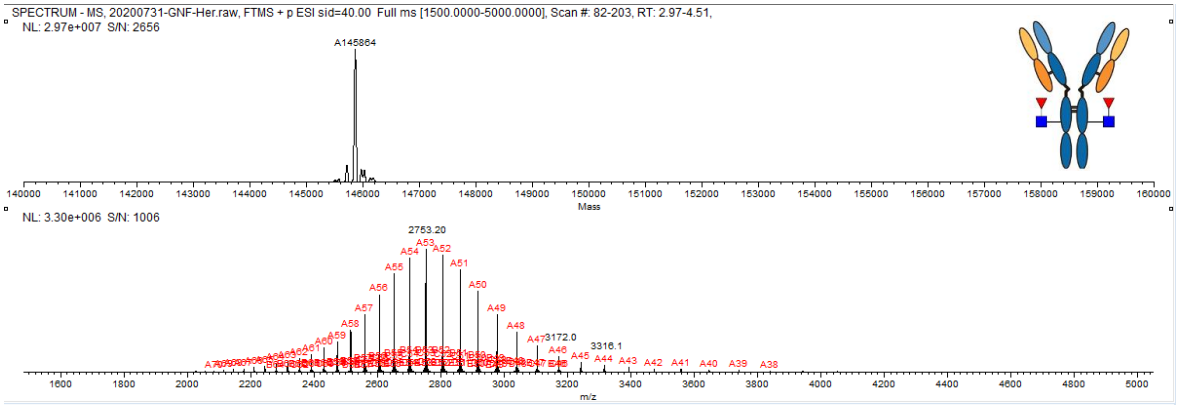

SPECTRUM - MS, 20200731-GNF-Her.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000], Scan #: 91-174, RT: 3.09-4.13,
NL: 3.88e+007 S/N: 3307
A145864
Mass
NL: 4.33e+006 S/N: 1788
2753.20
3172.0
3316.1
m/z

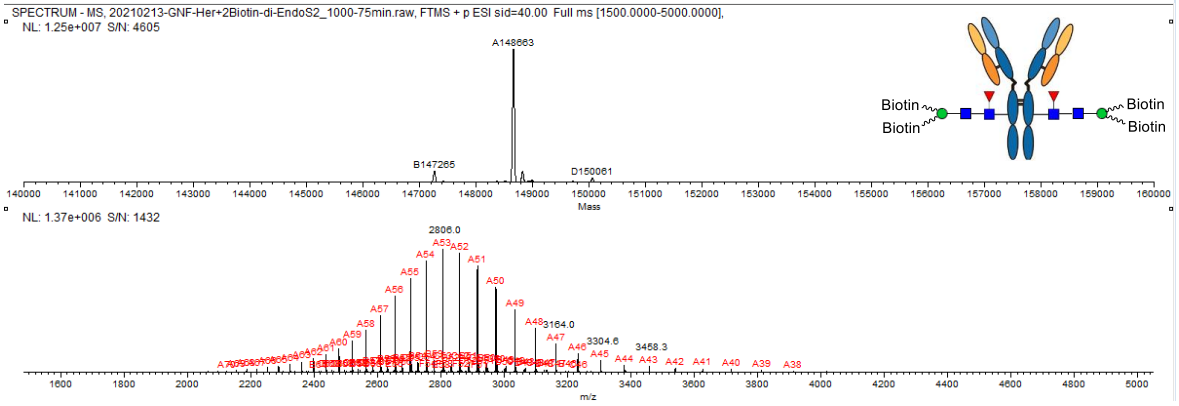

SPECTRUM - MS, 20210223-GNF-Her+2N3-Tetra-EndoS2-mutant-100-25min.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000],
NL: 1.23e+007 S/N: 2091
A147346
Mass
NL: 1.26e+006 S/N: 843
2781.17
3483136.0
3275.4
3427.6
3594.8
m/z

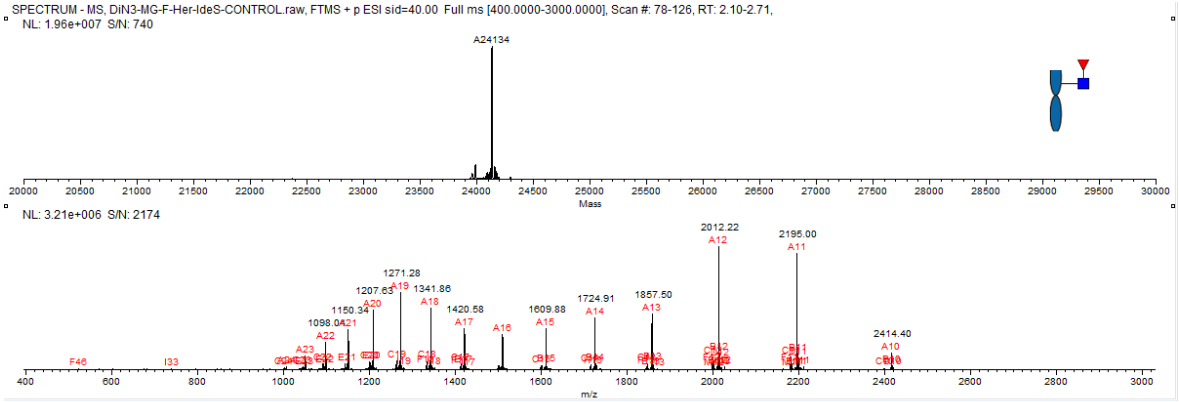

SPECTRUM - MS, DiN3-MG-F-Her-IdeS-CONTROL.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 80-127, RT: 2.13-2.72,
NL: 1.98e+007 S/N: 491
A24134
Mass
NL: 3.28e+006 S/N: 2219
2012.16
2195.03
1271.29
1207.64
1341.83
1150.32
1420.59
1609.90
1724.86
1857.52
2414.32
m/z

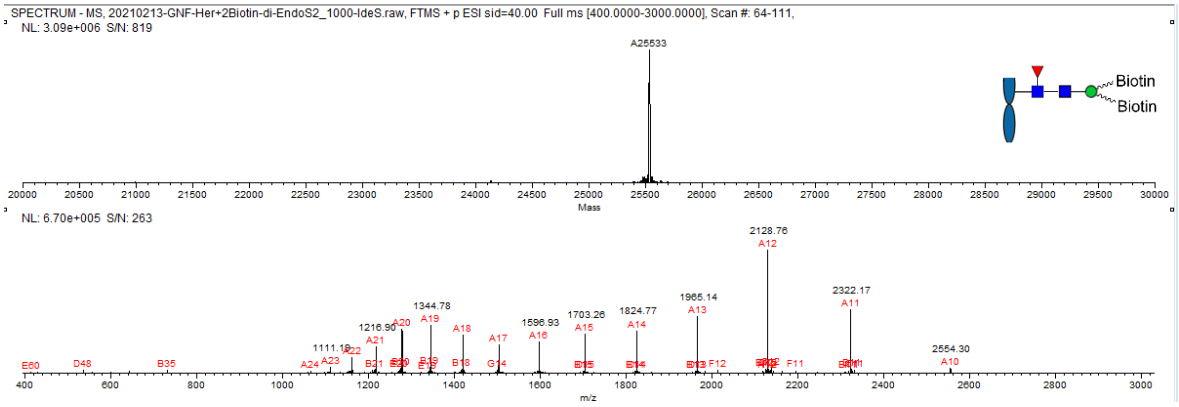

SPECTRUM - MS, 20210223-GNF-Her+2N3-Tetra-EndoS2-mutant-100-IdeS.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000],
NL: 4.42e+005 S/N: 524
A24874
C24135
Mass
NL: 9.33e+004 S/N: 125
2073.85
2262.32
1310.15
1185.50
1659.32
1777.72
1914.33
1555.67
2012.27
2195.24
2488.50
413.27
m/z

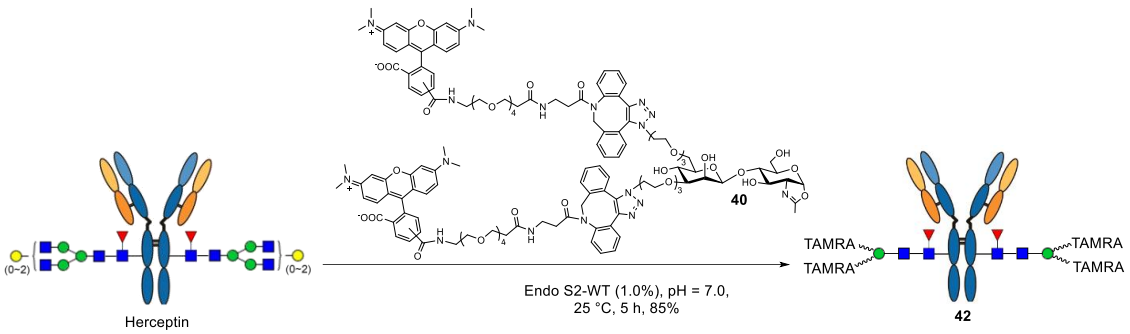

(0~2)
(0~2)
Herceptin
39
Endo S2-WT (0.1%), pH = 7.0),
25 °C, 1.5 h, 95%
Biotin
Biotin
Biotin
Biotin
41

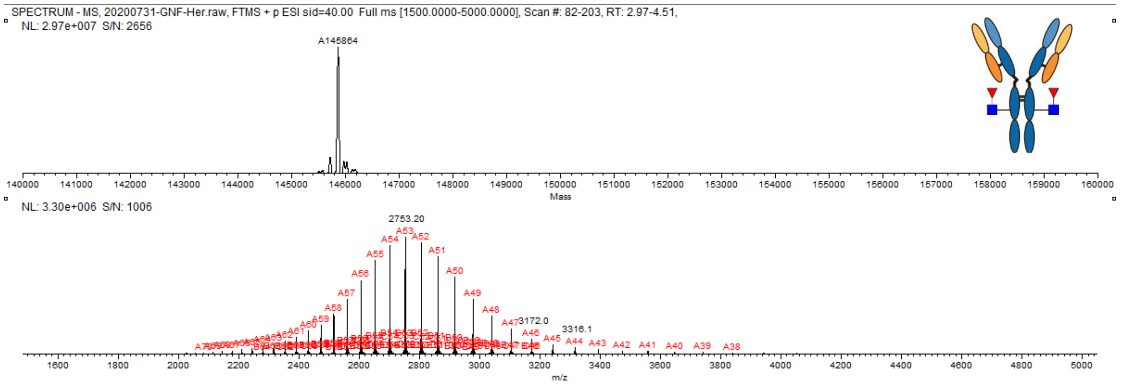

SPECTRUM - MS, 20200731-GNF-Her.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000], Scan #: 82-203, RT: 2.97-4.51,
NL: 2.97e+007 S/N: 2656
A145864
Mass
NL: 3.30e+006 S/N: 1006
2753.20
3172.0
3316.1
m/z

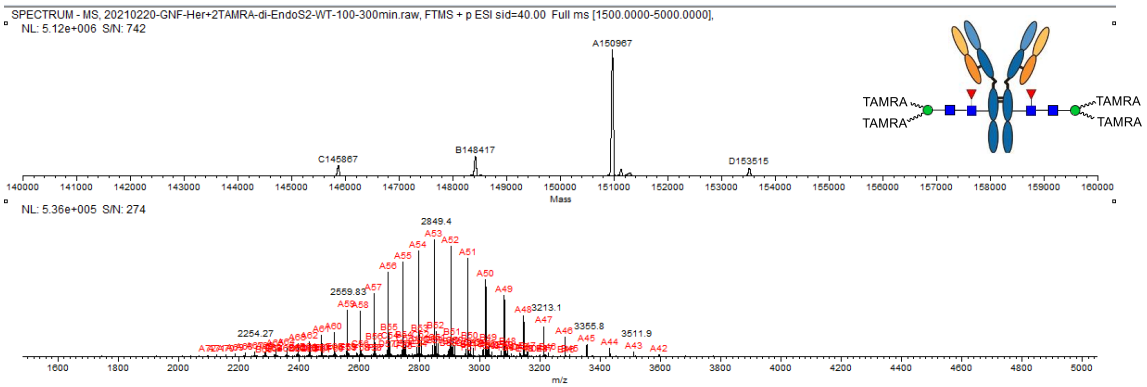

SPECTRUM - MS, 20210213-GNF-Her+2Biotin-di-EndoS2_1000-75min.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000],
NL: 1.25e+007 S/N: 4605
A148663
B147265
D150061
Biotin
Biotin
Biotin
Biotin
Mass
NL: 1.37e+006 S/N: 1432
2806.0
3164.0
3304.6
3458.3
m/z

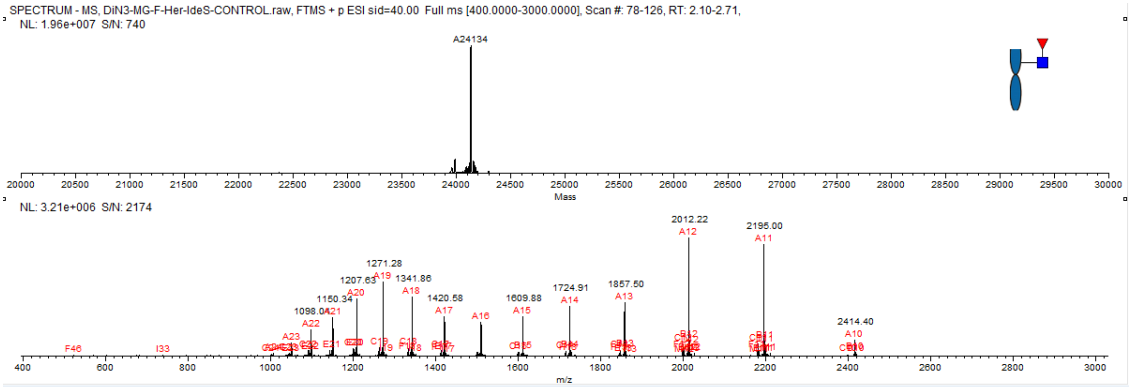

SPECTRUM - MS, DiN3-MG-F-Her-IdeS-CONTROL.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 78-126, RT: 2.10-2.71,
NL: 1.96e+007 S/N: 740
A24134
Mass
NL: 3.21e+006 S/N: 2174
2012.22
2195.00
1271.28
1207.63
1341.86
1150.34
1420.58
1098.04
1609.88
1724.91
1857.50
2414.40
m/z

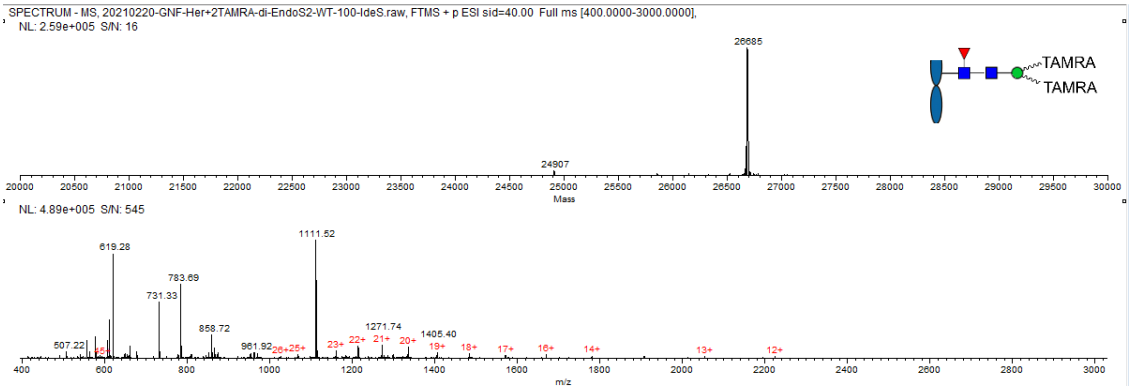

SPECTRUM - MS, 20210213-GNF-Her+2Biotin-di-EndoS2_1000-IdeS.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 64-111,
NL: 3.09e+006 S/N: 819
A25533
Biotin
Biotin
Mass
NL: 6.70e+005 S/N: 263
2128.76
2322.17
1344.78
1216.90
1596.93
1703.26
1824.77
1965.14
2554.30
m/z


(0–2)
(0–2)
Herceptin
40
Endo S2-WT (1.0%), pH = 7.0,
25 °C, 5 h, 85%
TAMRA
TAMRA
TAMRA
TAMRA
42

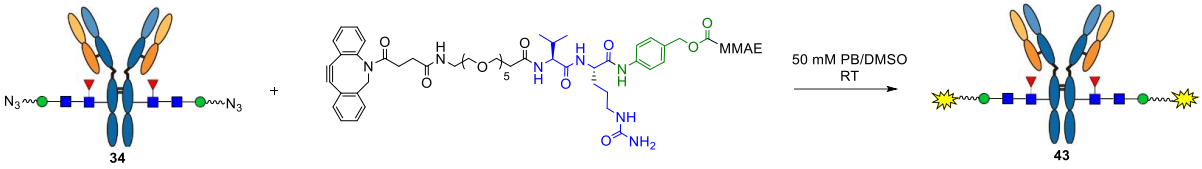

SPECTRUM - MS, 20200731-GNF-Her.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000], Scan #: 82-203, RT: 2.97-4.51,
NL: 2.97e+007 S/N: 2656
A145864
Mass
NL: 3.30e+006 S/N: 1006
2753.20
3172.0
3316.1
m/z

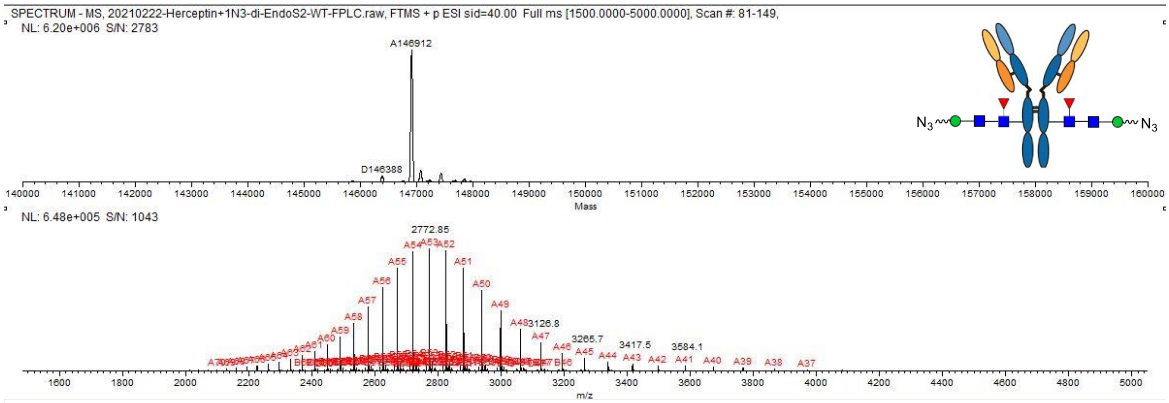

SPECTRUM - MS, 20210220-GNF-Her+2TAMRA-di-EndoS2-WT-100-300min.raw, FTMS + p ESI sid=40.00 Full ms [1500.0000-5000.0000],
NL: 5.12e+006 S/N: 742
A150967
C145867
B148417
D153515
TAMRA
TAMRA
TAMRA
TAMRA
Mass
NL: 5.36e+005 S/N: 274
2849.4
2559.93
2254.27
3213.1
3355.8
3511.9
m/z

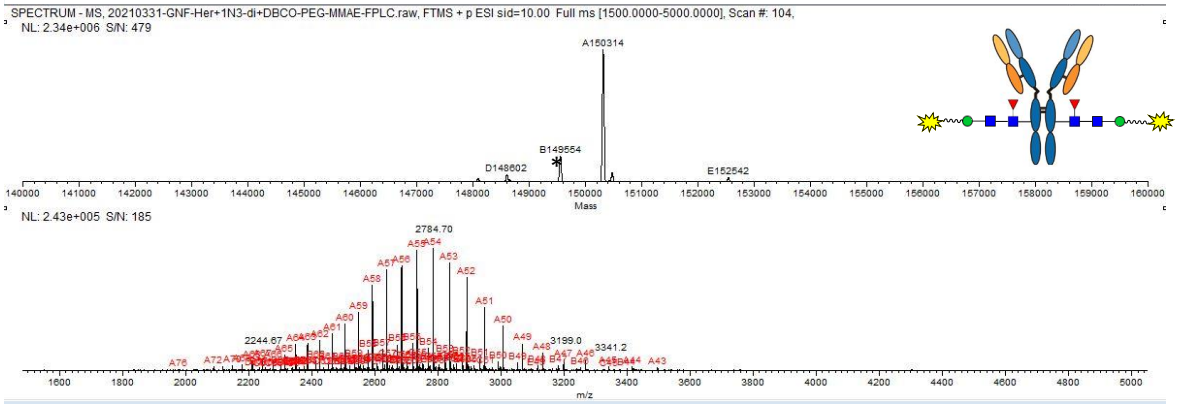

SPECTRUM - MS, DiN3-MG-F-Her-IdeS-CONTROL.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 78-126, RT: 2.10-2.71,
NL: 1.96e+007 S/N: 740
A24134
Mass
NL: 3.21e+006 S/N: 2174
2012.22
2195.00
1271.28
1207.65
1341.86
1150.34
1420.58
1098.04
1609.88
1724.91
1857.50
2414.40
m/z

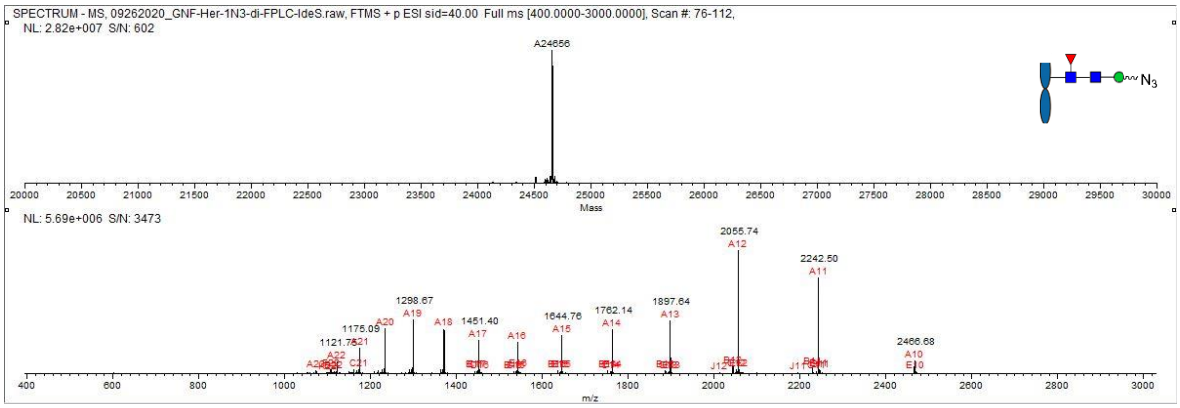

SPECTRUM - MS, 20210220-GNF-Her+2TAMRA-di-EndoS2-WT-100-IdeS.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000],
NL: 2.59e+005 S/N: 16
26685
24907
TAMRA
TAMRA
Mass
NL: 4.89e+005 S/N: 545
1111.52
619.28
783.69
731.33
858.72
1271.74
1405.40
507.22
961.92
m/z

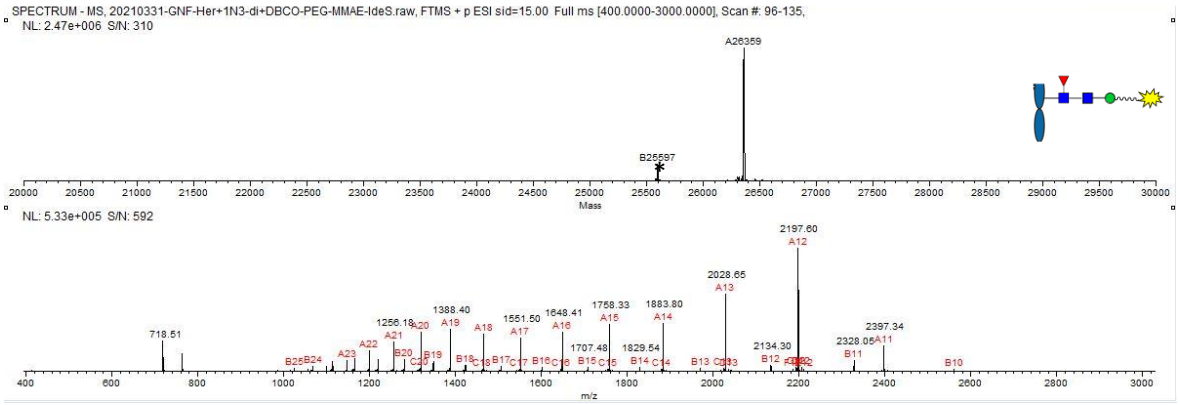
4. LC-MS analysis of the homogenous ADCs.

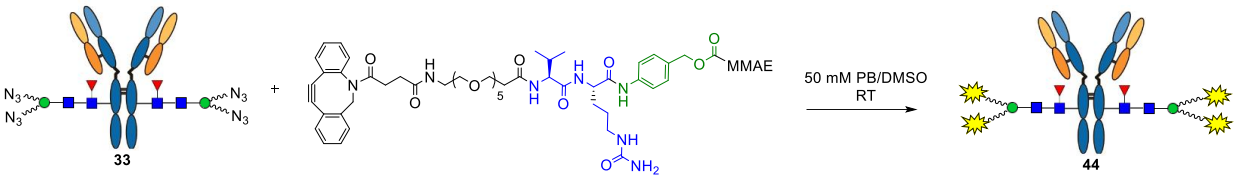


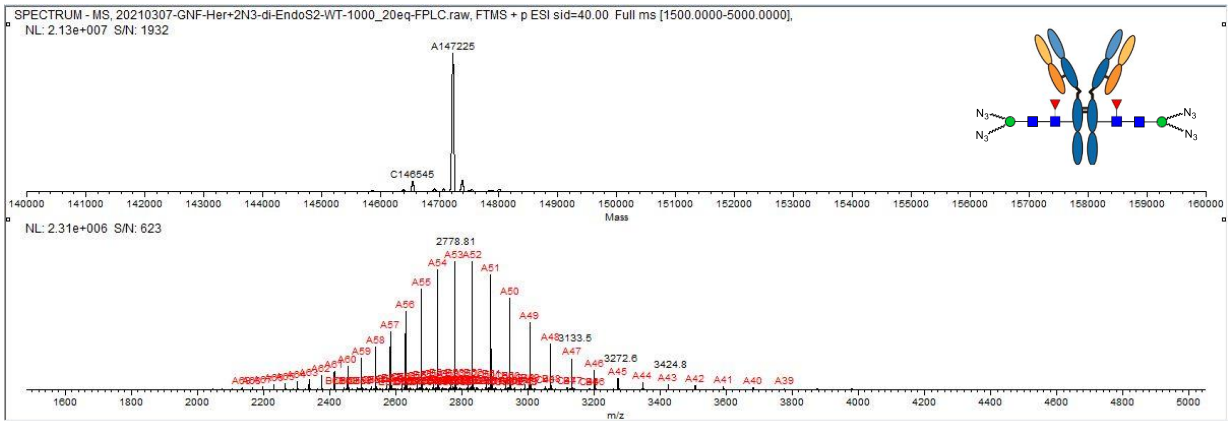









33
MMAE
50 mM PB/DMSO
RT
44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NL: 2.13e+007 S/N: 1932
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C146545
Mass
NL: 2.31e+006 S/N: 623
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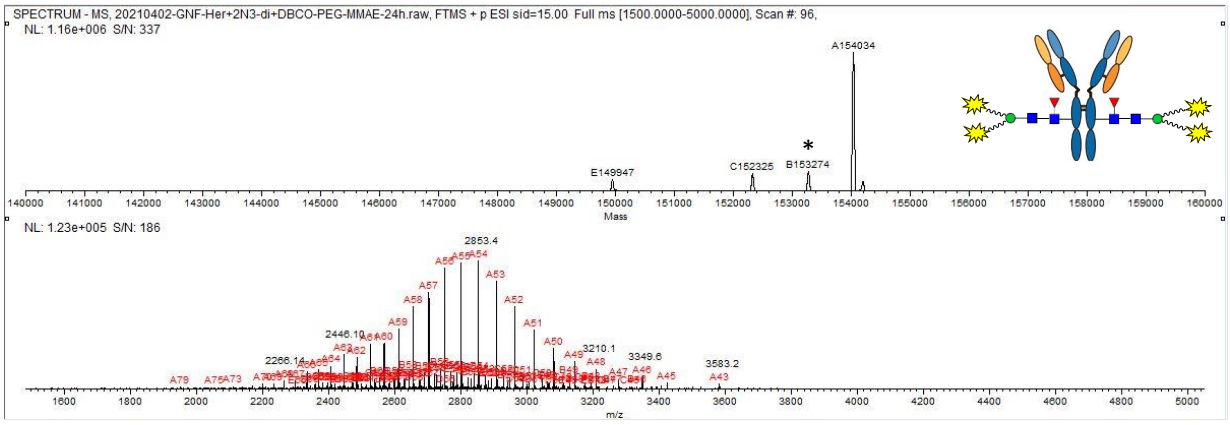
3133.5
3272.6
3424.8
m/z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NL: 1.16e+006 S/N: 337
A154034
E149947
C152325
B153274
Mass
NL: 1.23e+005 S/N: 186
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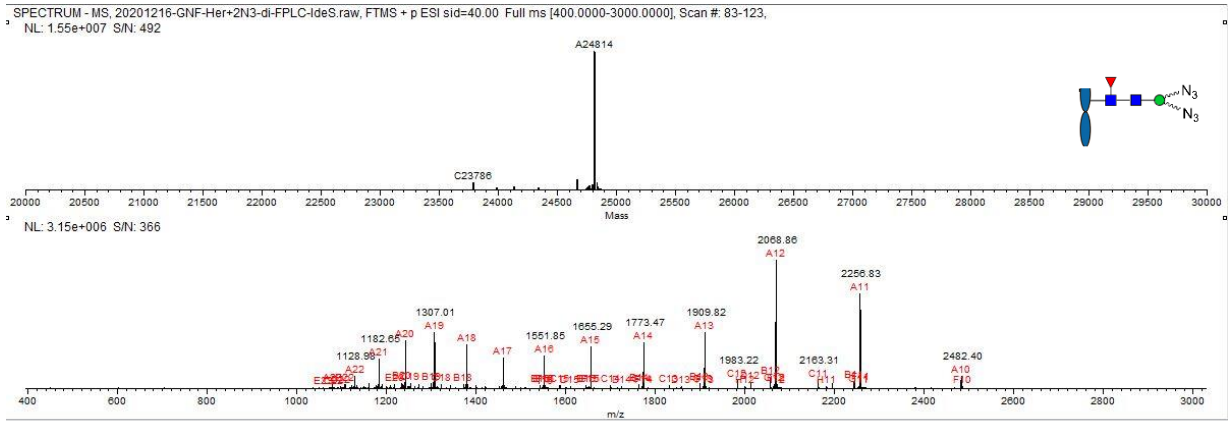
3210.1
3349.6
3583.2
m/z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C23786
Mass
NL: 3.15e+006 S/N: 366
2068.86
2256.83
1307.01
1909.82
1655.29
1773.47
1551.85
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1128.98
1983.22
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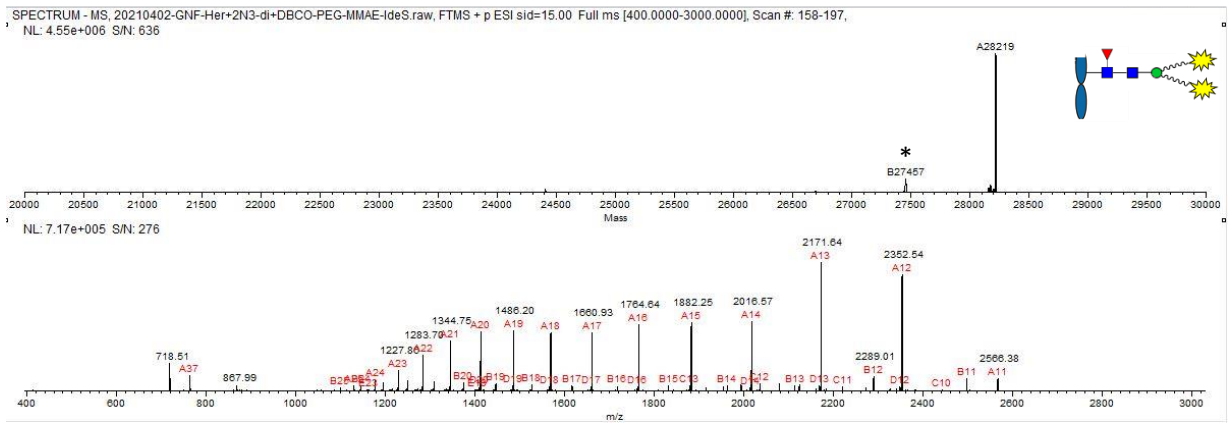
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A28219
B27457
Mass
NL: 7.17e+005 S/N: 276
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2352.54
1486.20
1660.93
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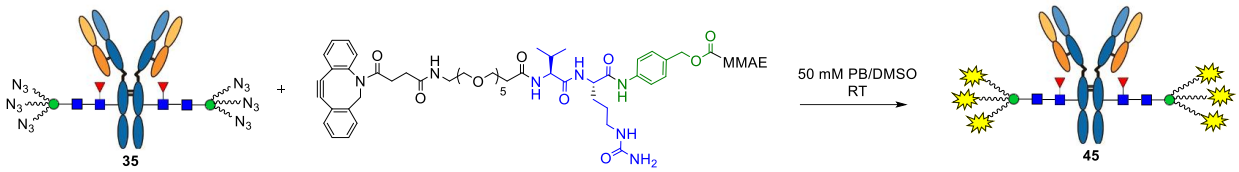
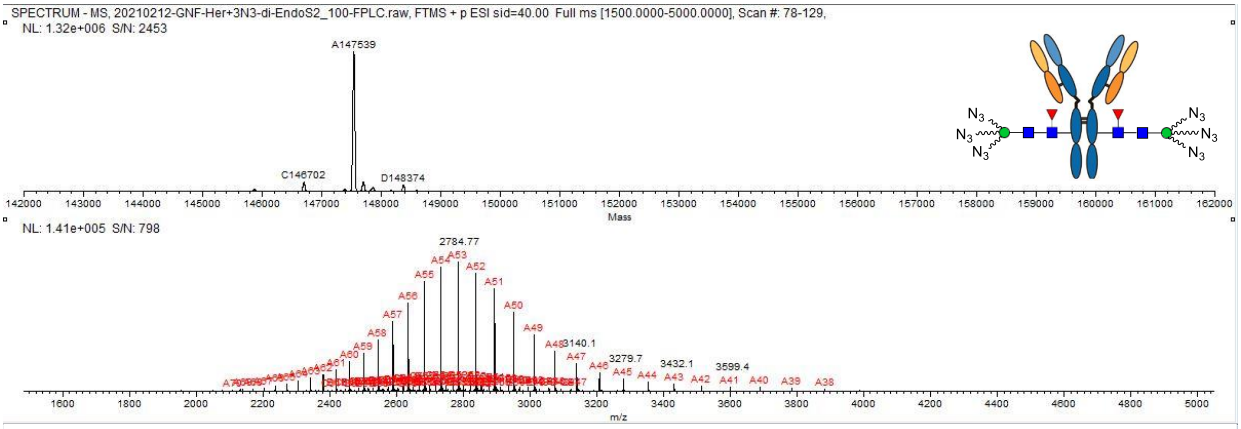
1344.75
1283.70
1227.86
718.51
867.99
2289.01
2566.38
m/z


35
MMAE
50 mM PB/DMSO
RT
45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NL: 1.32e+006 S/N: 2453
A147539
C146702
D148374
Mass
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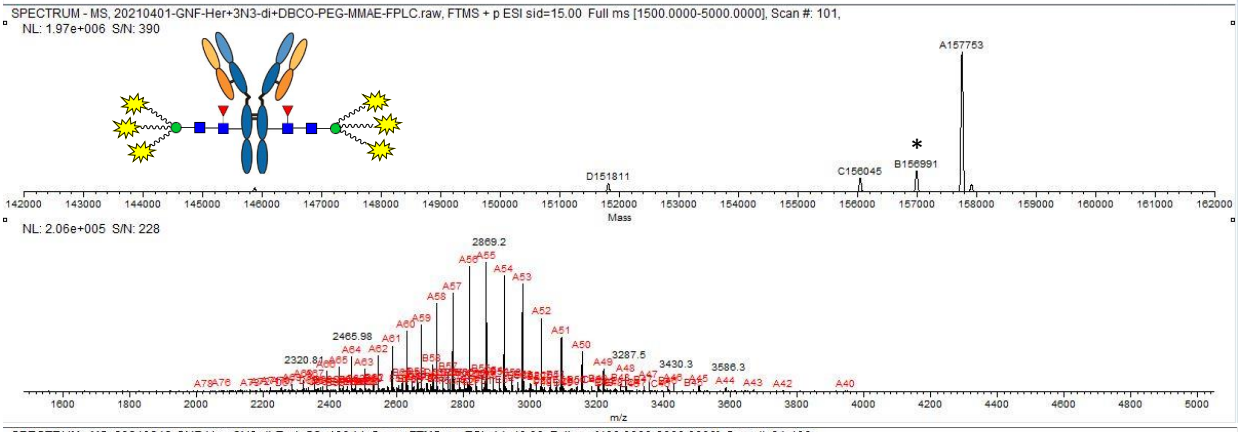
3140.1
3279.7
3432.1
3599.4
m/z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NL: 1.97e+006 S/N: 390
A157753
D151811
C156045
B156991
Mass
NL: 2.06e+005 S/N: 228
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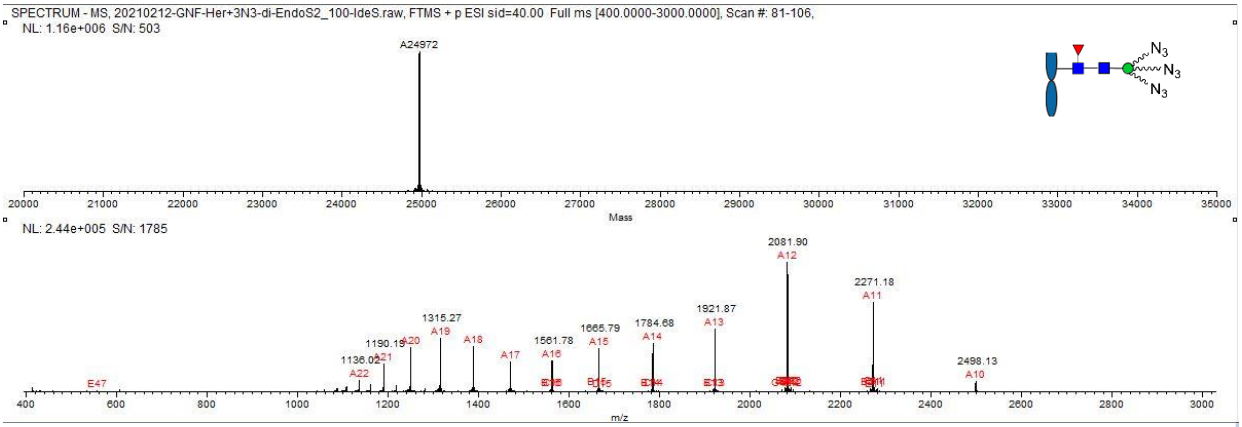
2465.98
2320.81
3287.5
3430.3
3586.3
m/z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NL: 1.16e+006 S/N: 503
A24972
Mass
NL: 2.44e+005 S/N: 1785
2081.90
2271.18
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1784.68
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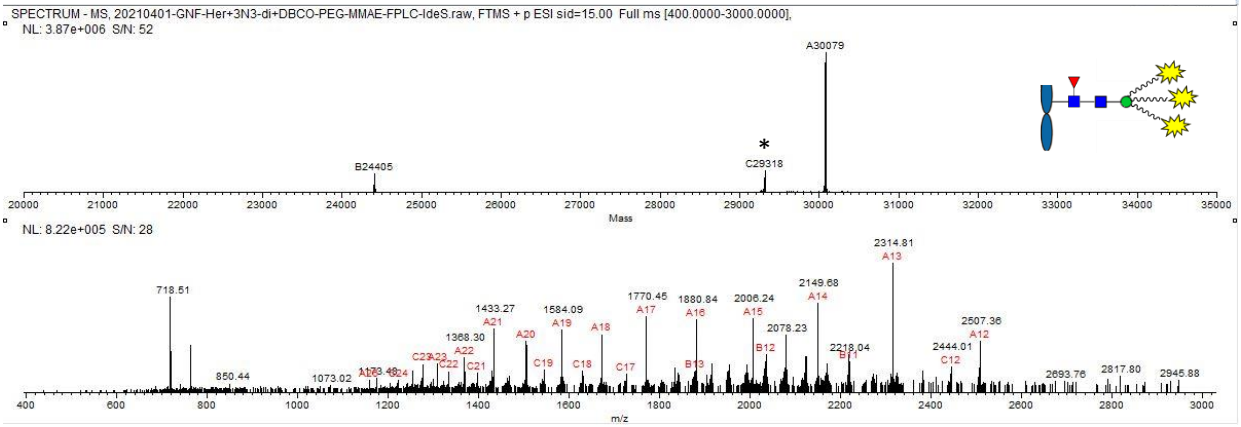
1315.27
1190.15
1136.02
2498.13
m/z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NL: 3.87e+006 S/N: 52
A30079
B24405
C29318
Mass
NL: 8.22e+005 S/N: 28
2314.81
718.51
2149.68
1770.45
1880.84
2006.24
1584.09
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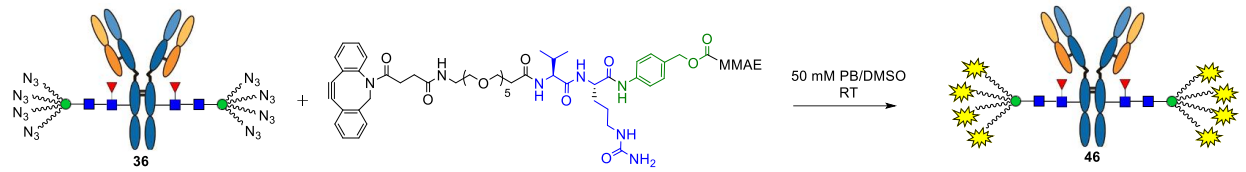
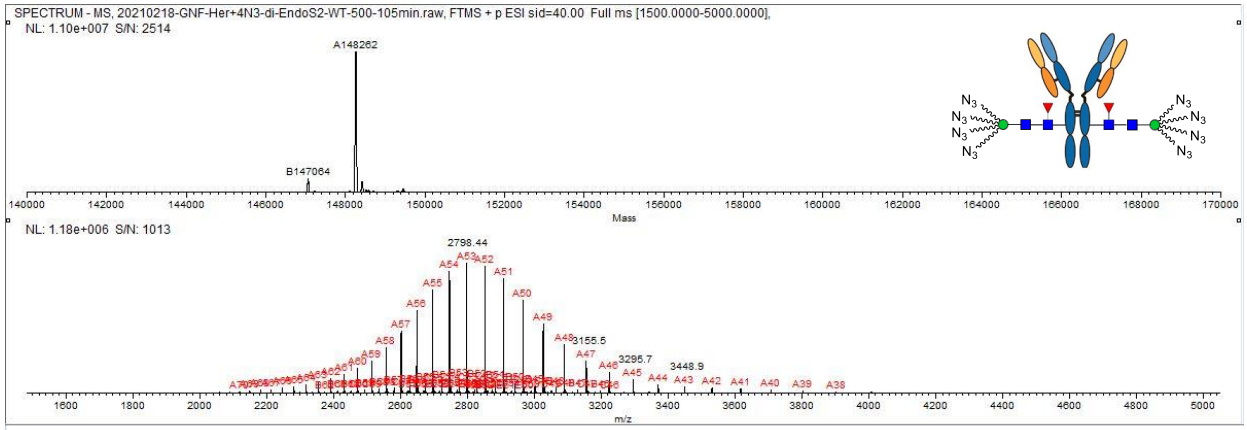
1368.30
2078.23
2507.36
2444.01
2693.76
2817.80
2945.88
m/z


36
MMAE
50 mM PB/DMSO
RT
46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A148262
B147064
Mass
NL: 1.18e+006 S/N: 1013
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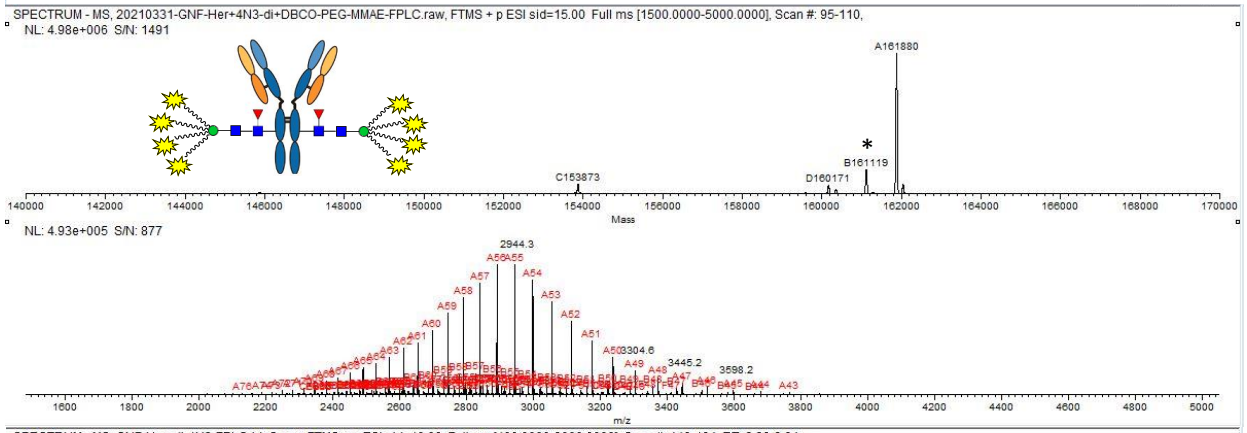
3155.5
3295.7
3448.9
m/z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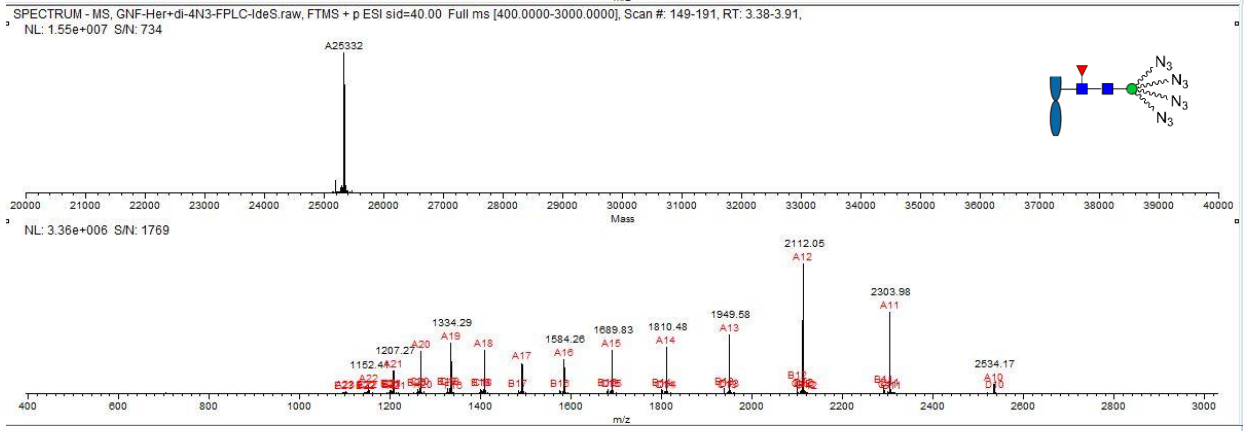
NL: 4.98e+006 S/N: 1491
A161880
B161119
C153873
D160171
Mass
NL: 4.93e+005 S/N: 877
2944.3
3304.6
3445.2
3598.2
m/z


SPECTRUM - MS, GNF-Her+di-4N3-FPLC-IdeS.raw, FTMS + p ESI sid=40.00 Full ms [400.0000-3000.0000], Scan #: 149-191, RT: 3.38-3.91,
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A25332
Mass
NL: 3.36e+006 S/N: 1769
2112.05
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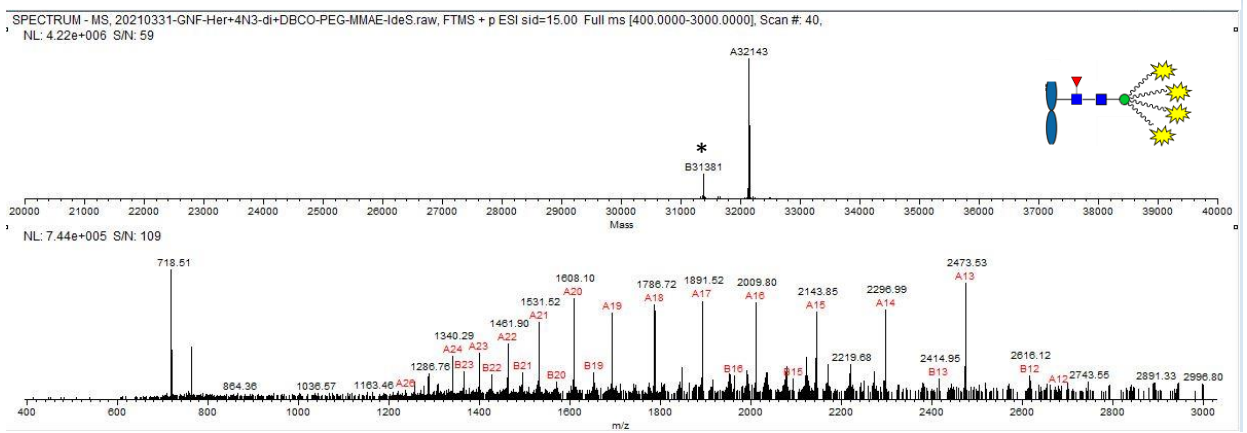
1207.27
1152.44
2534.17
m/z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NL: 4.22e+006 S/N: 59
A32143
B31381
Mass
NL: 7.44e+005 S/N: 109
718.51
2473.53
1608.10
1786.72
1891.52
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2143.85
2296.99
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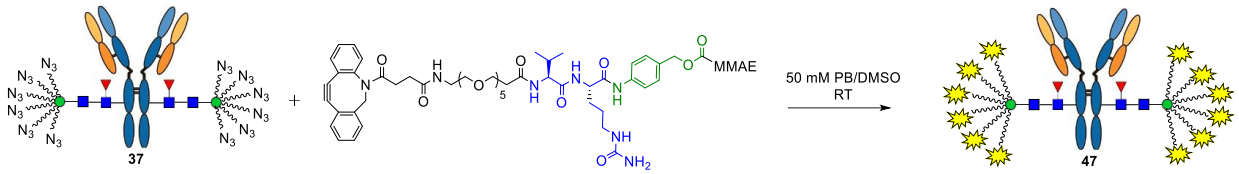
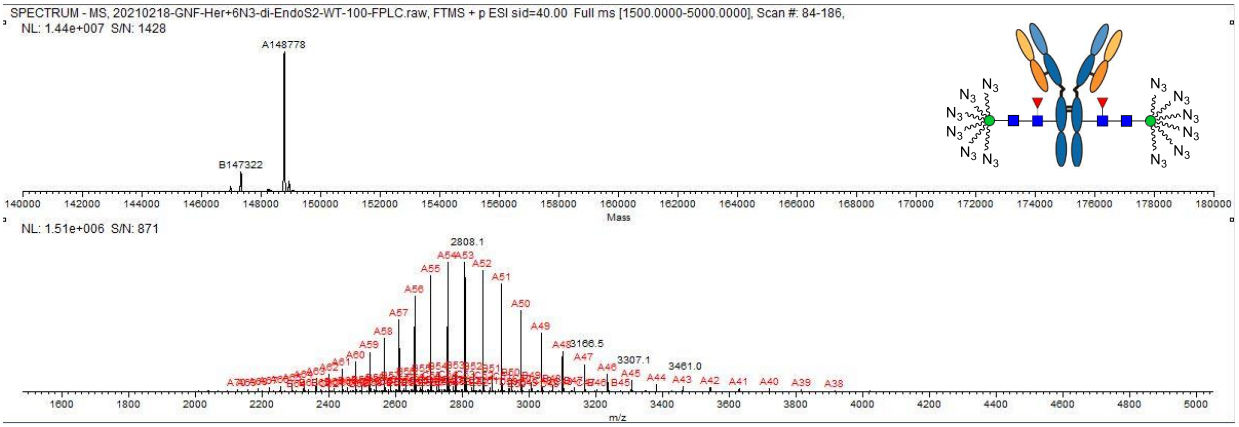
2996.80
m/z


37
MMAE
50 mM PB/DMSO
RT
47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B147322
Mass
NL: 1.51e+006 S/N: 871
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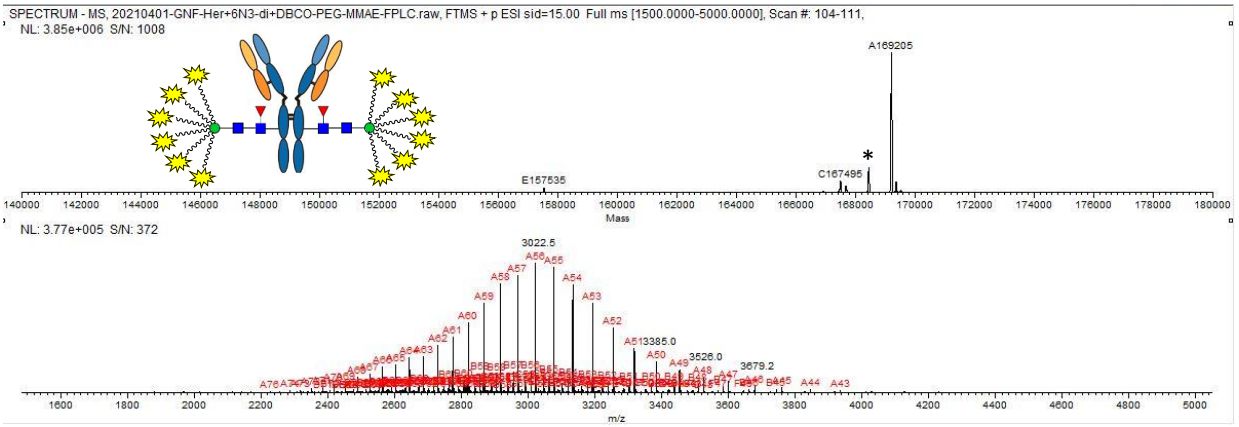
3166.5
3307.1
3461.0
m/z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A169205
E157535
C167495
Mass
NL: 3.77e+005 S/N: 372
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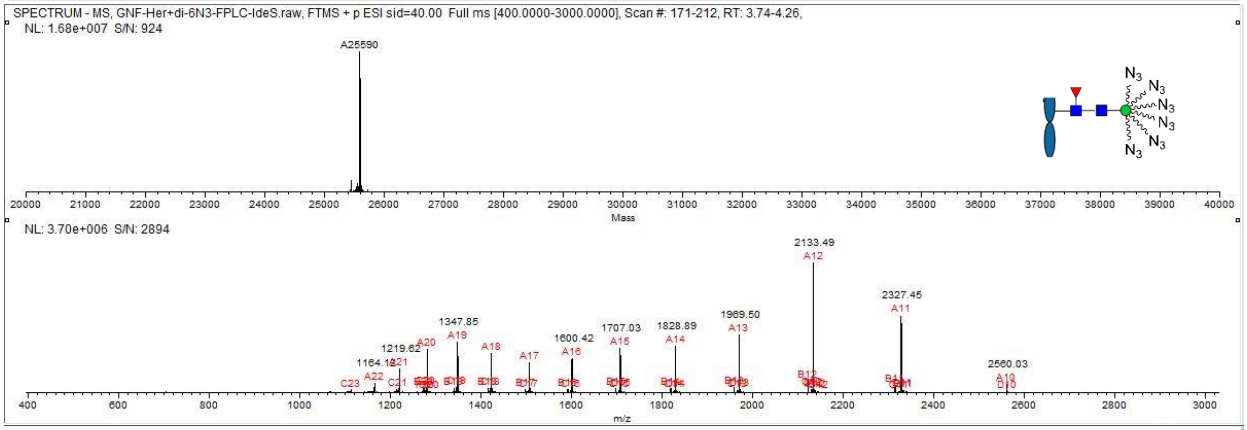
3526.0
3679.2
m/z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A25590
Mass
NL: 3.70e+006 S/N: 2894
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1969.50
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1707.03
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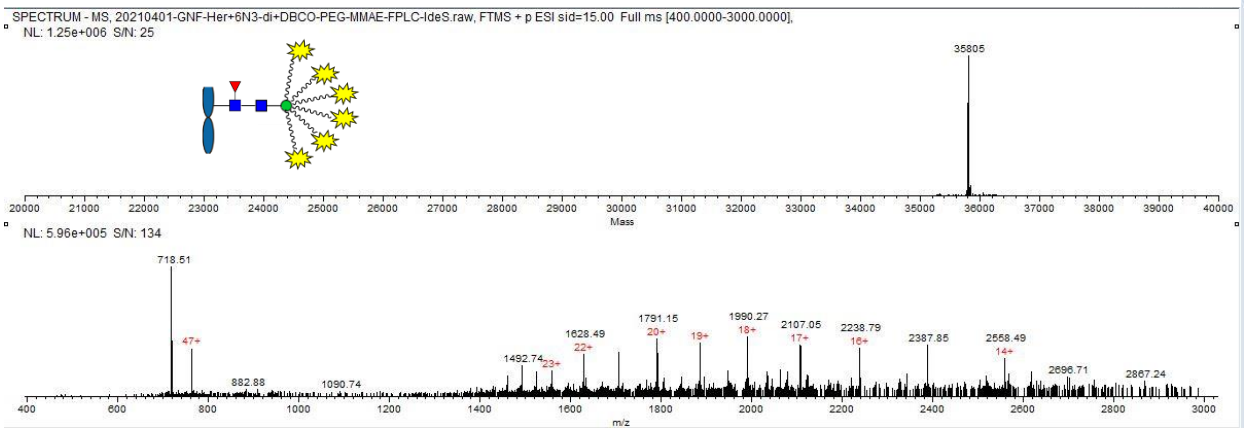
1347.85
1219.62
2560.03
m/z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35805
Mass
NL: 5.96e+005 S/N: 134
718.51
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2558.49
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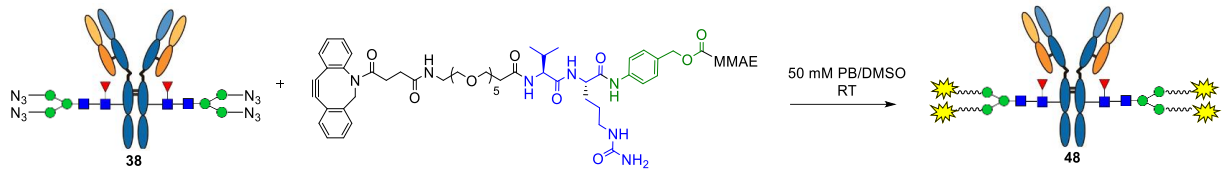
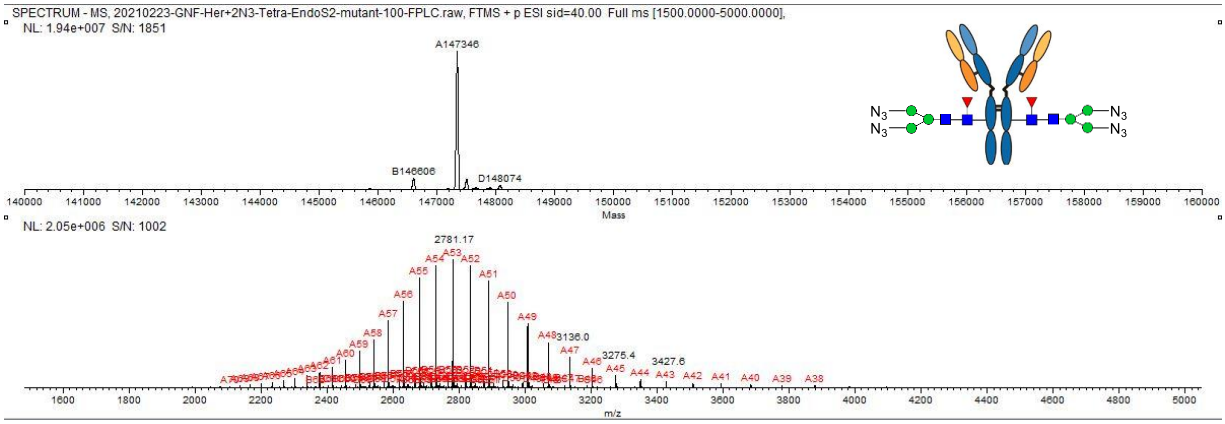
2867.24
m/z


38
MMAE
50 mM PB/DMSO
RT
48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A147346
B146606
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Mass
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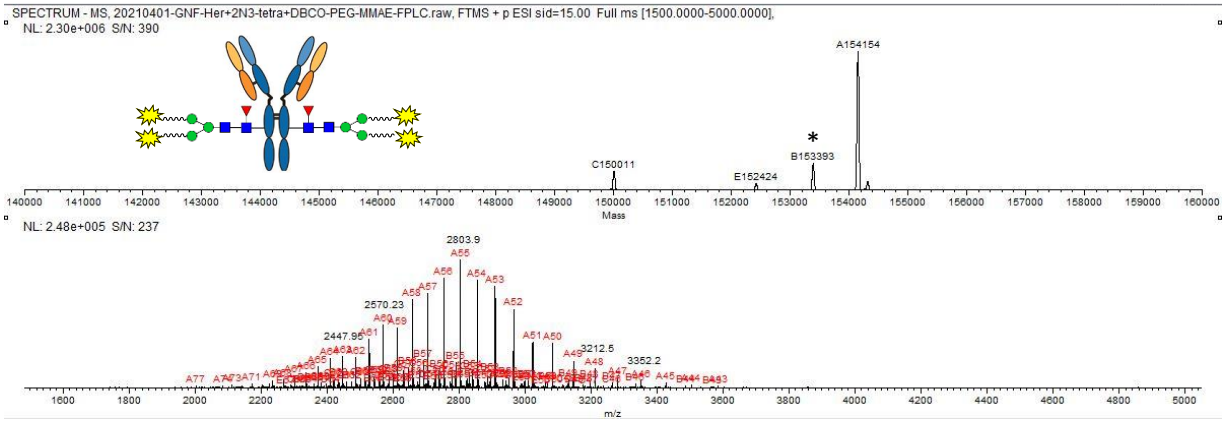
3136.0
3275.4
3427.6
m/z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NL: 2.30e+006 S/N: 390
A154154
B153393
C150011
E152424
Mass
NL: 2.48e+005 S/N: 237
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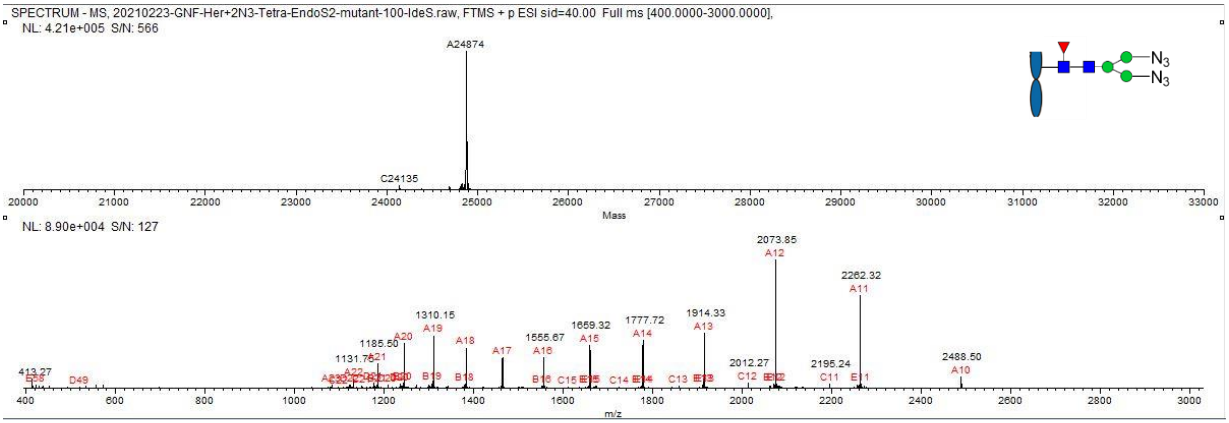
2447.96
3212.5
3352.2
m/z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A24874
C24135
Mass
NL: 8.90e+004 S/N: 127
2073.85
2262.32
1310.15
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1659.32
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2195.24
2488.50
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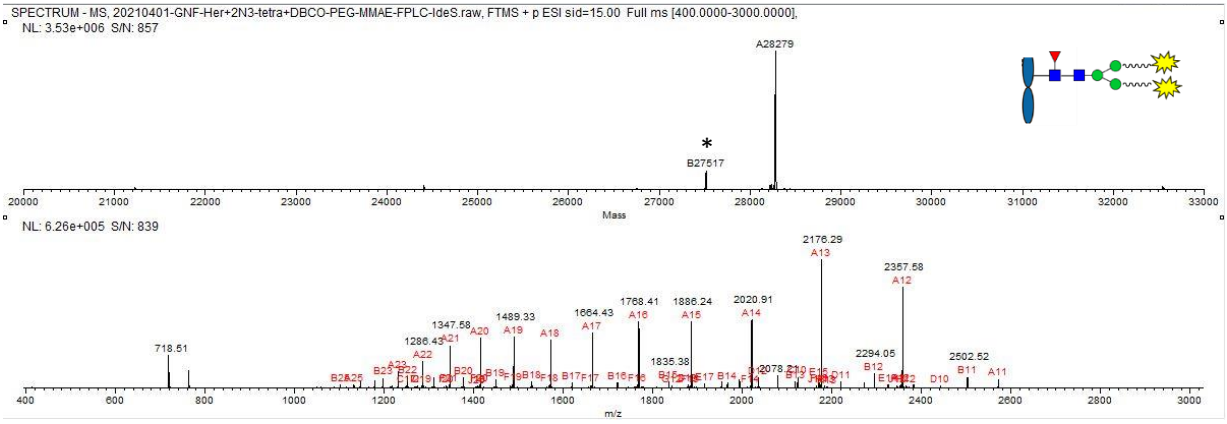
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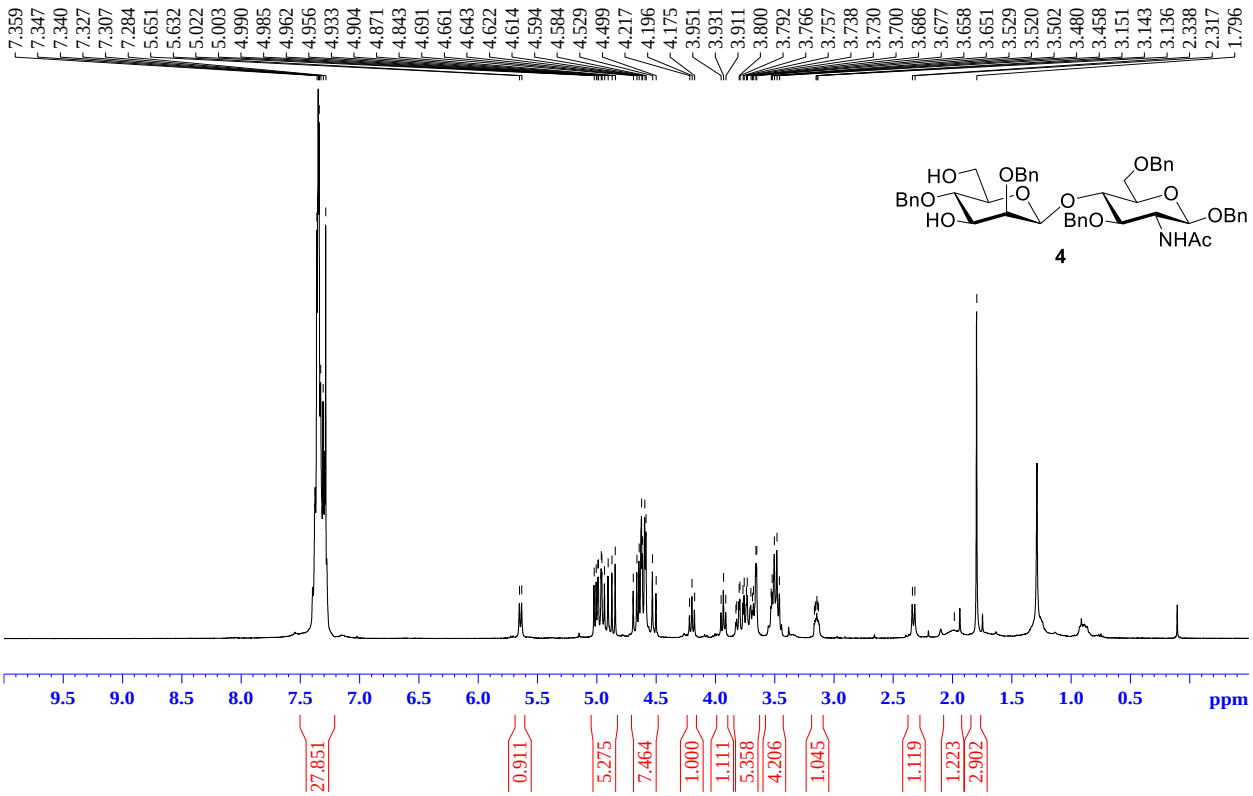
A28279
B27517
Mass
NL: 6.26e+005 S/N: 839
2176.29
2357.58
1768.41
1886.24
2020.91
1664.43
1489.33
1347.58
1286.43
718.51
1835.38
2294.05
2502.52
m/z

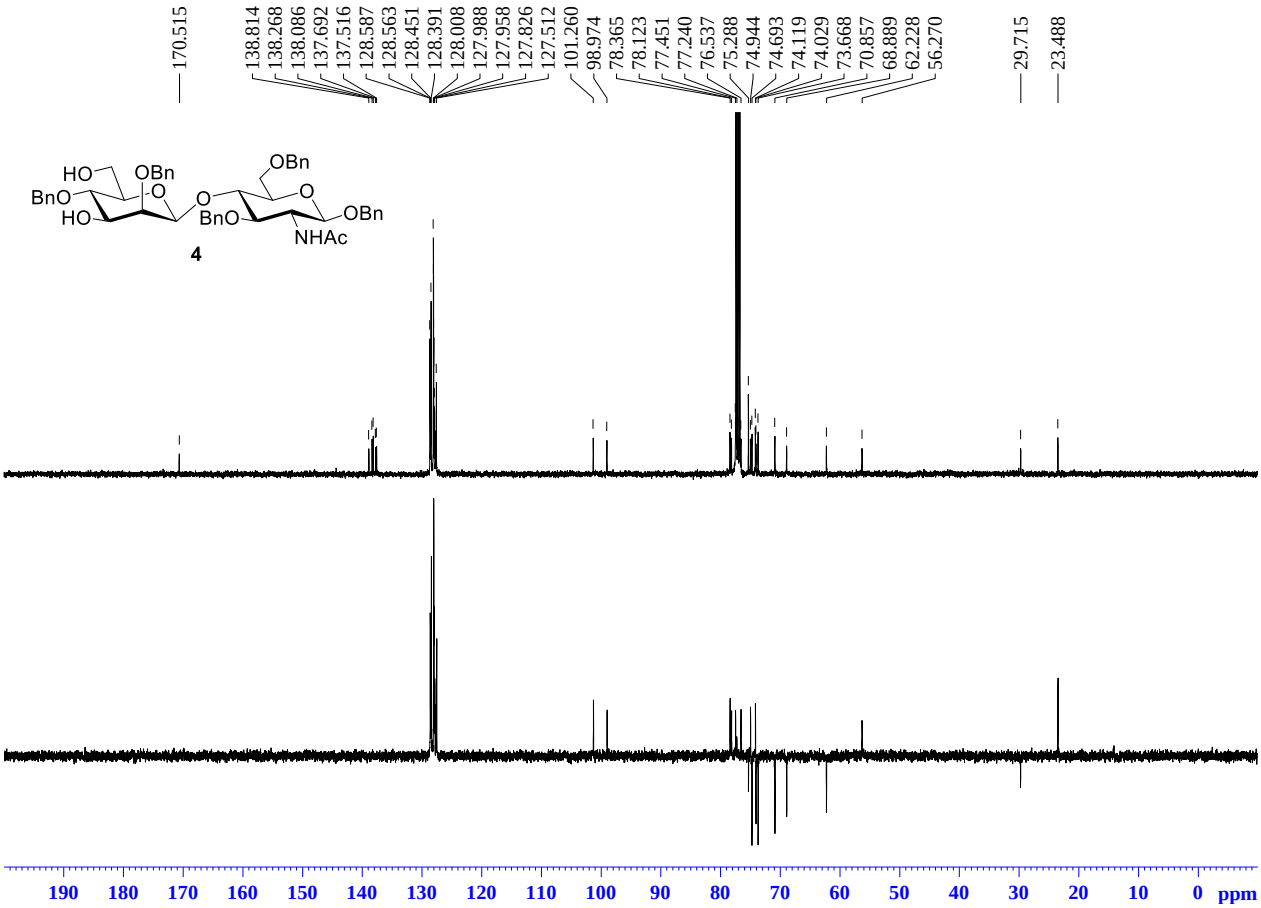
5. References:

1. H. Ochiai, W. Huang, L.-X. Wang, Expeditious chemoenzymatic synthesis of homogeneous N-glycoproteins carrying defined oligosaccharide ligands, *J Am Chem Soc*, **2008**, *130*, 13790-13803.
2. A. Lossouarn, K. Renault, L. Bailly, A. Frisby, P. L. Nahenec-Martel, P.-Y. Renard, C. Sabot, Maleimide-based metal-free ligation with dienes: a comparative study, *Org Biomol Chem*, **2020**, *18*, 3874-3887.
3. H. A Orgueira, A. Bartolozzi, P. Schell, R. E. J. N. Litjens, E. R. Palmacci, P. H. Seeberger, Modular Synthesis of Heparin Oligosaccharides, *Chem Eur J*, **2003**, *9*, 140-169.
4. B. Linclau, E. Cini, C. S. Oakes, S. Josse, M. Light, V. Ironmonger, Stereoarrays with an all-carbon quaternary center: diastereoselective desymmetrization of prochiral malonaldehydes, *Angew Chem Int Ed*, **2012**, *51*, 1232-1235.
5. W. Ji, L. Xiao, Y. Ling, C. Ching, M. Matsumoto, R. P. Bisbey, D. E. Helbling, W. R. Dichtel, *J Am Chem Soc*, **2018**, *140*, 12677-12681.
6. T. Kentaro, T. Taishi, H. Takeshi, S. Mitsuhiko, A Close-packed, Highly Insulating Organic Thin Monolayer on Si(111), *Chem Lett*, **2008**, *37*, 440-441.
7. C.-S. Kwan, A. S. C. Chan, K. C.-F. Leung, A Fluorescent and Switchable Rotaxane Dual Organocatalyst, *Org Lett*, **2016**, *18*, 976-979.

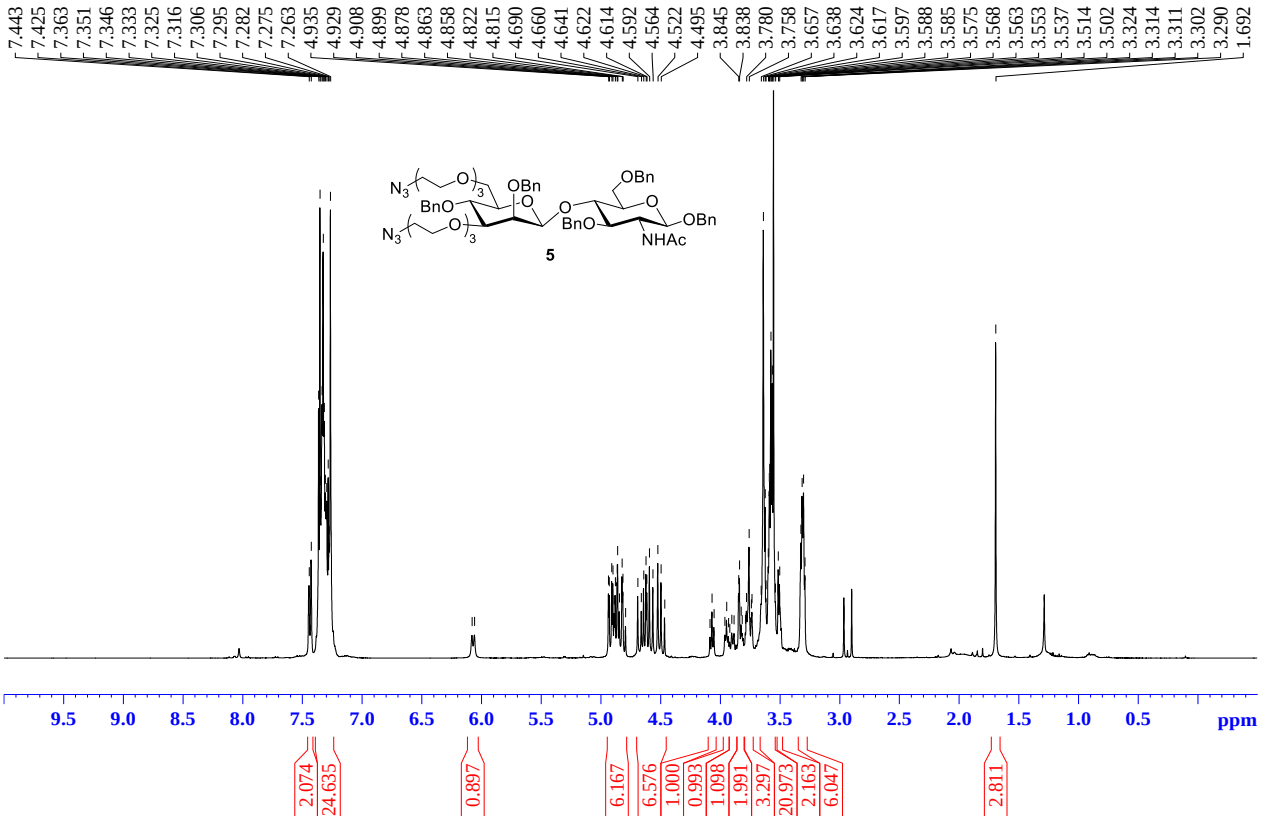
6. NMR Spectra



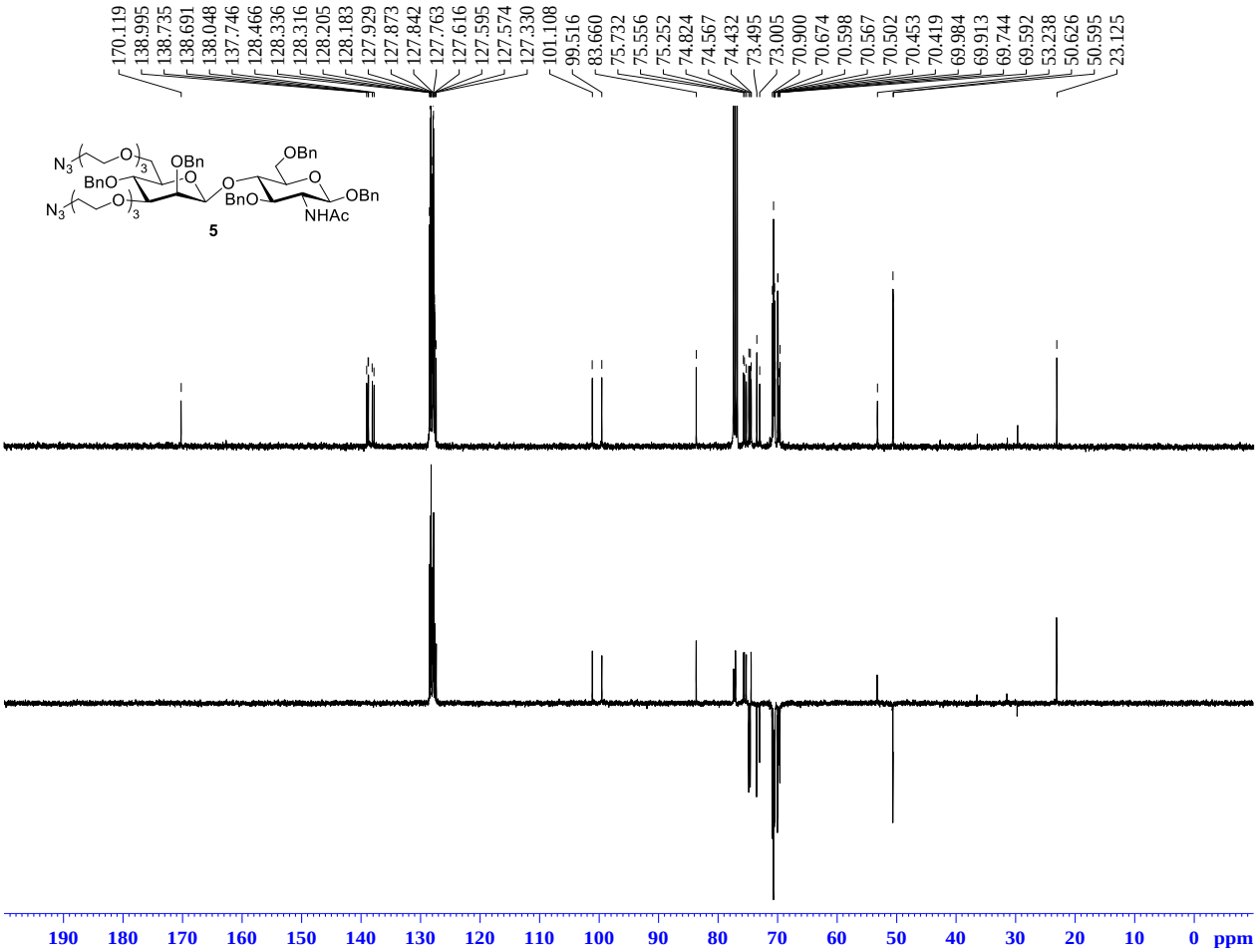
Compound 4: ^{1}H NMR ($CDCl_3$, 400 MHz)



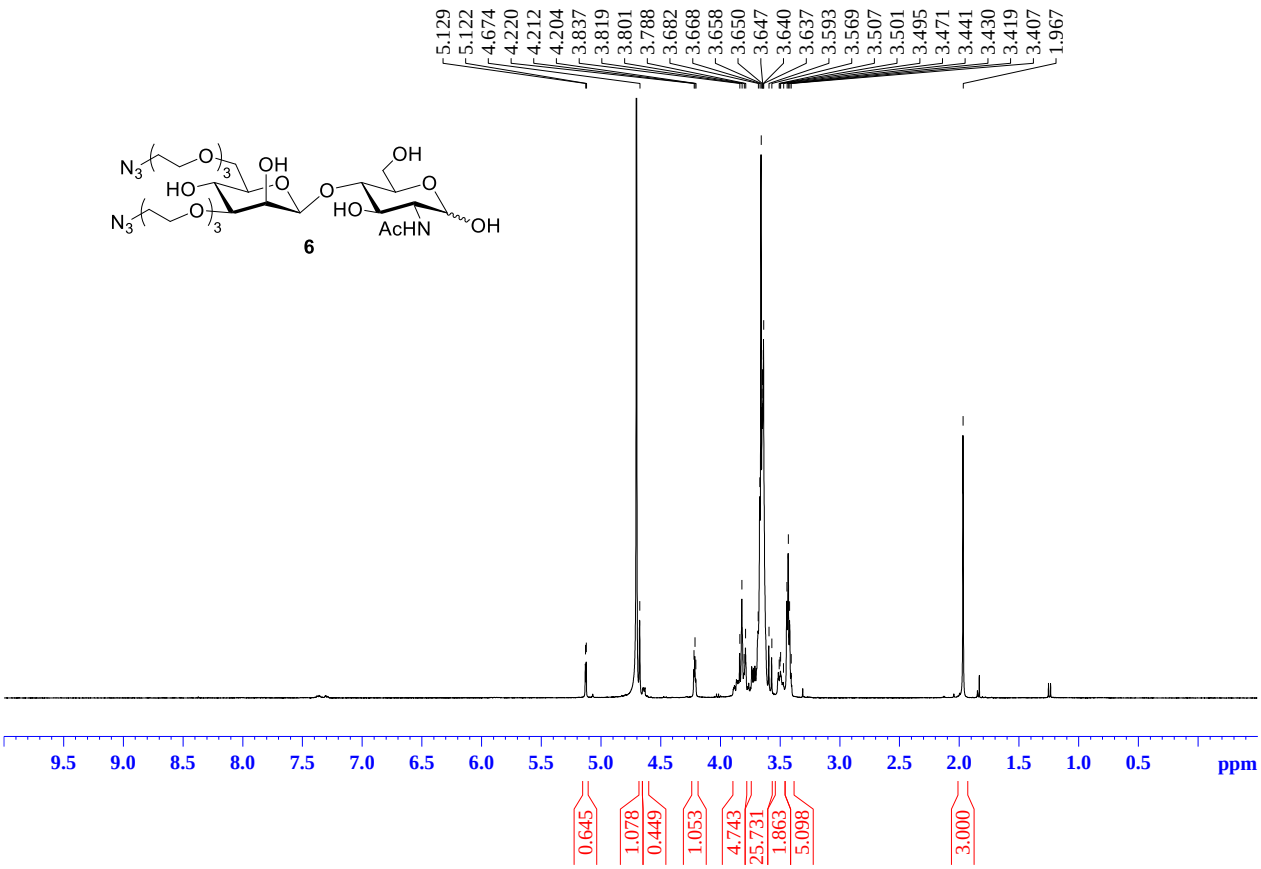
Compound 4: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



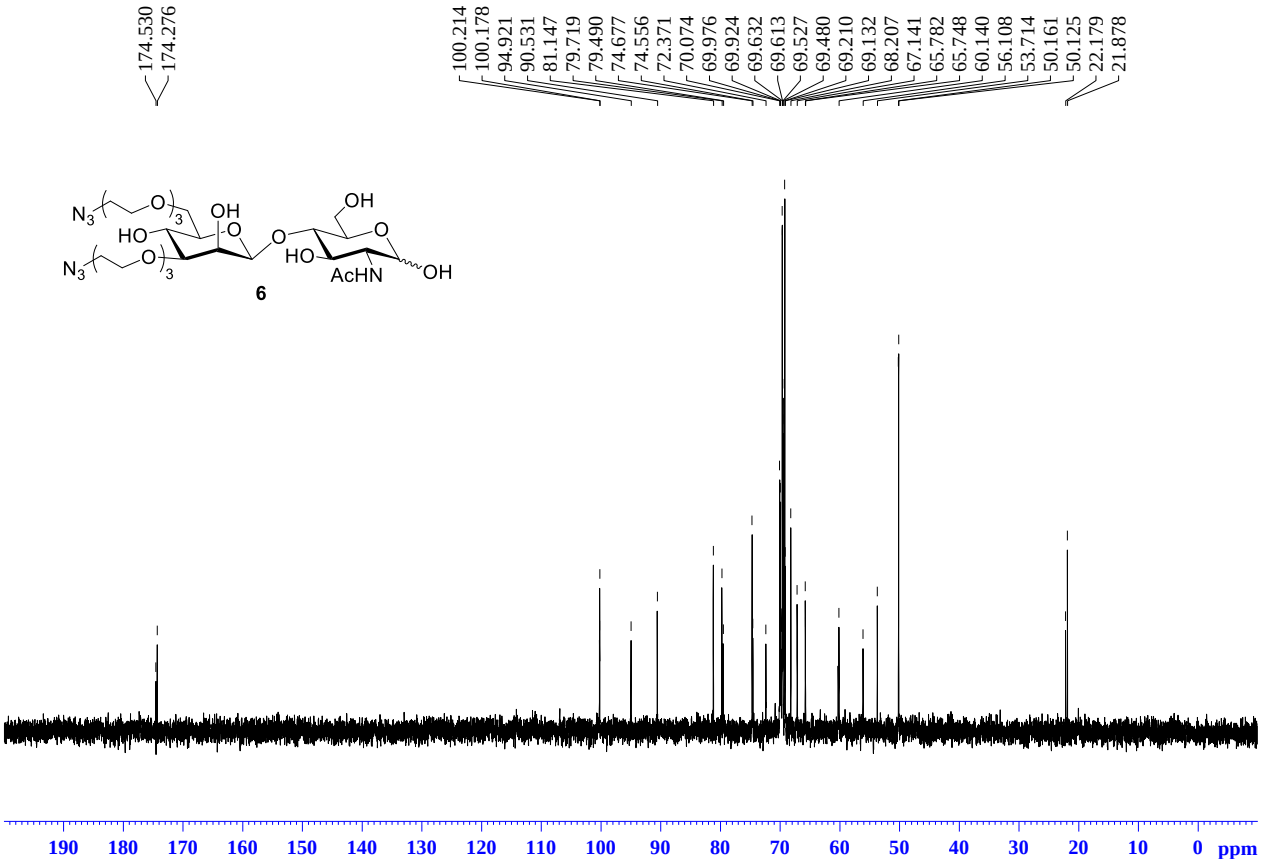
Compound 5: ^{1}H NMR ($CDCl_3$, 400 MHz)



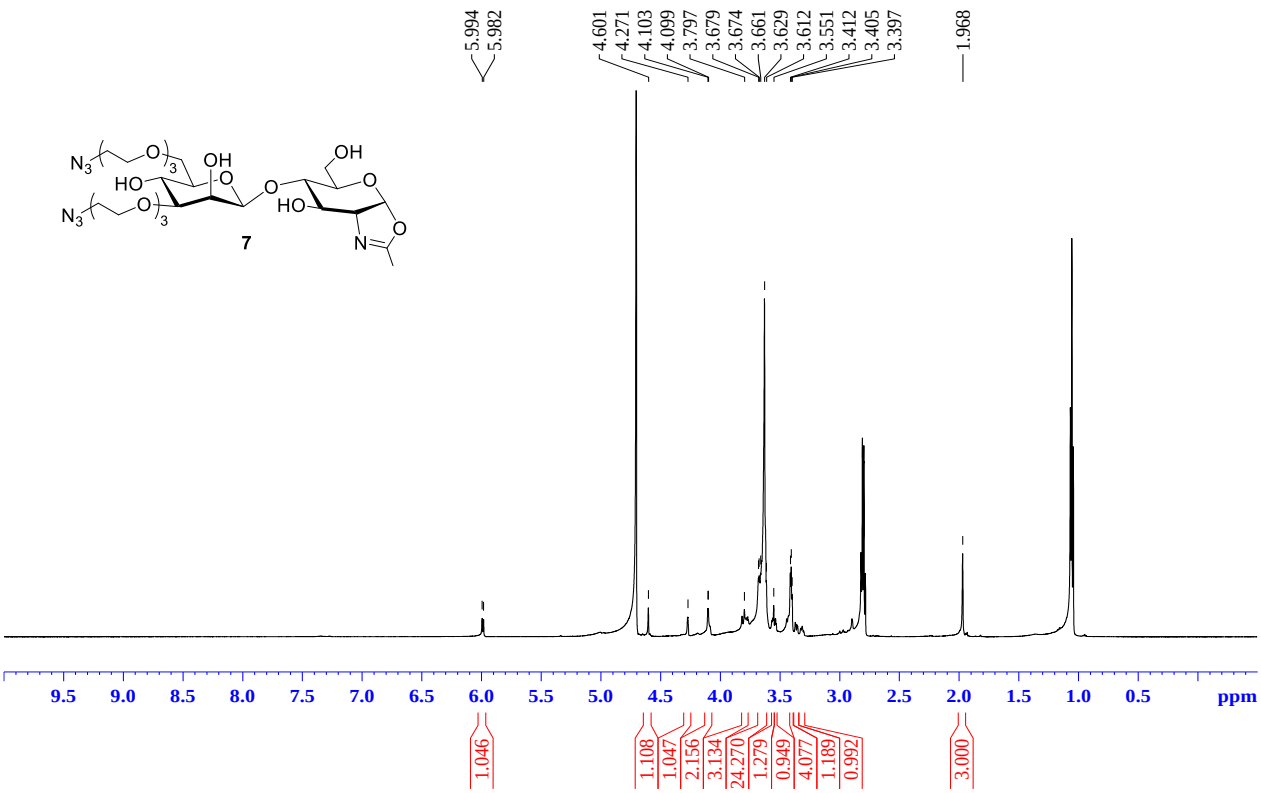
Compound 5: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



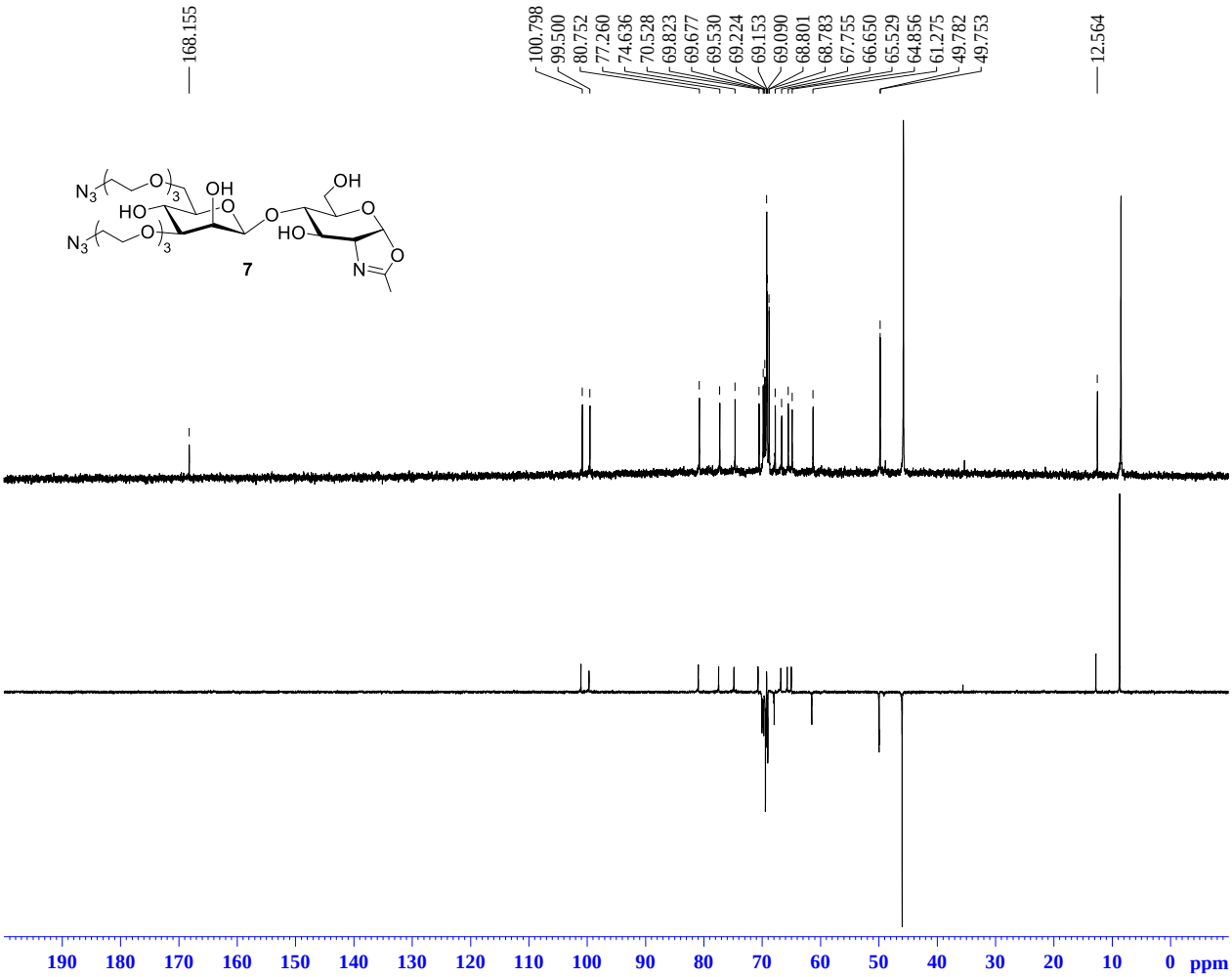
Compound 6: ^{1}H NMR (D_2O, 400 MHz)



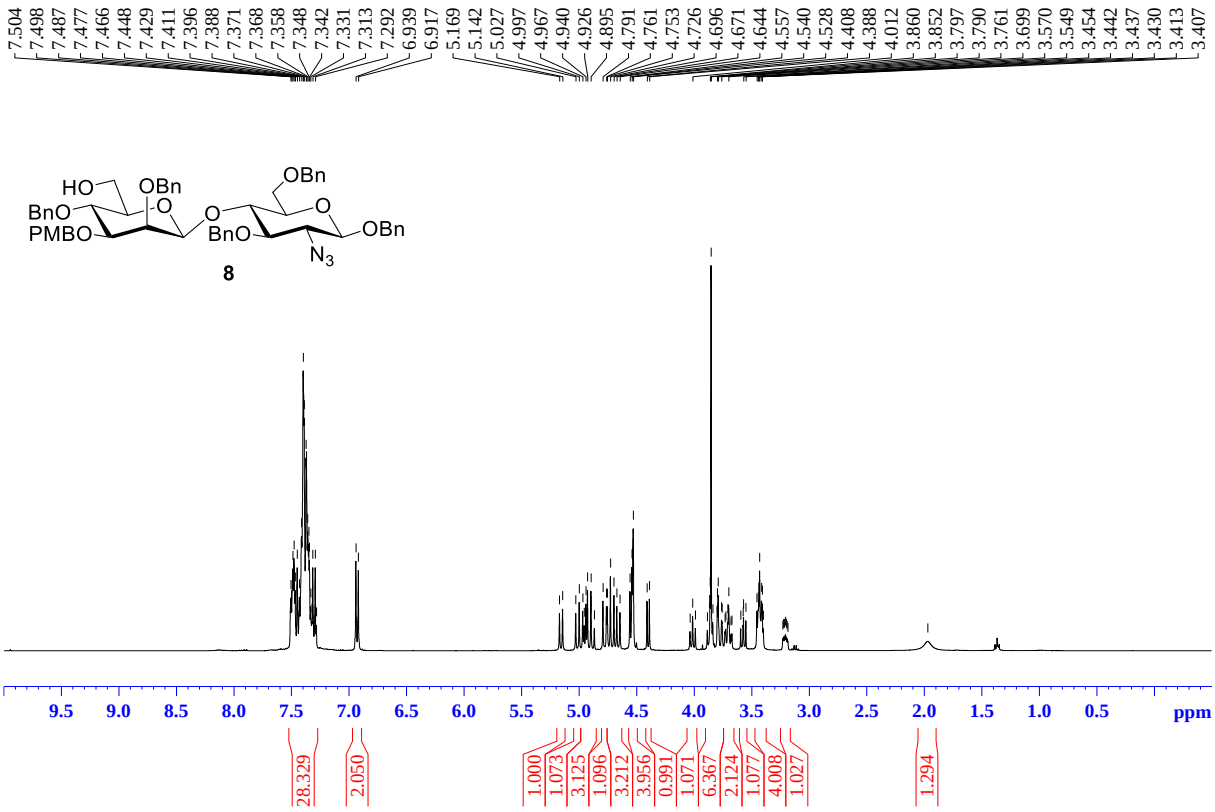
Compound 6: ^{13}C NMR (D_2O, 100 MHz)



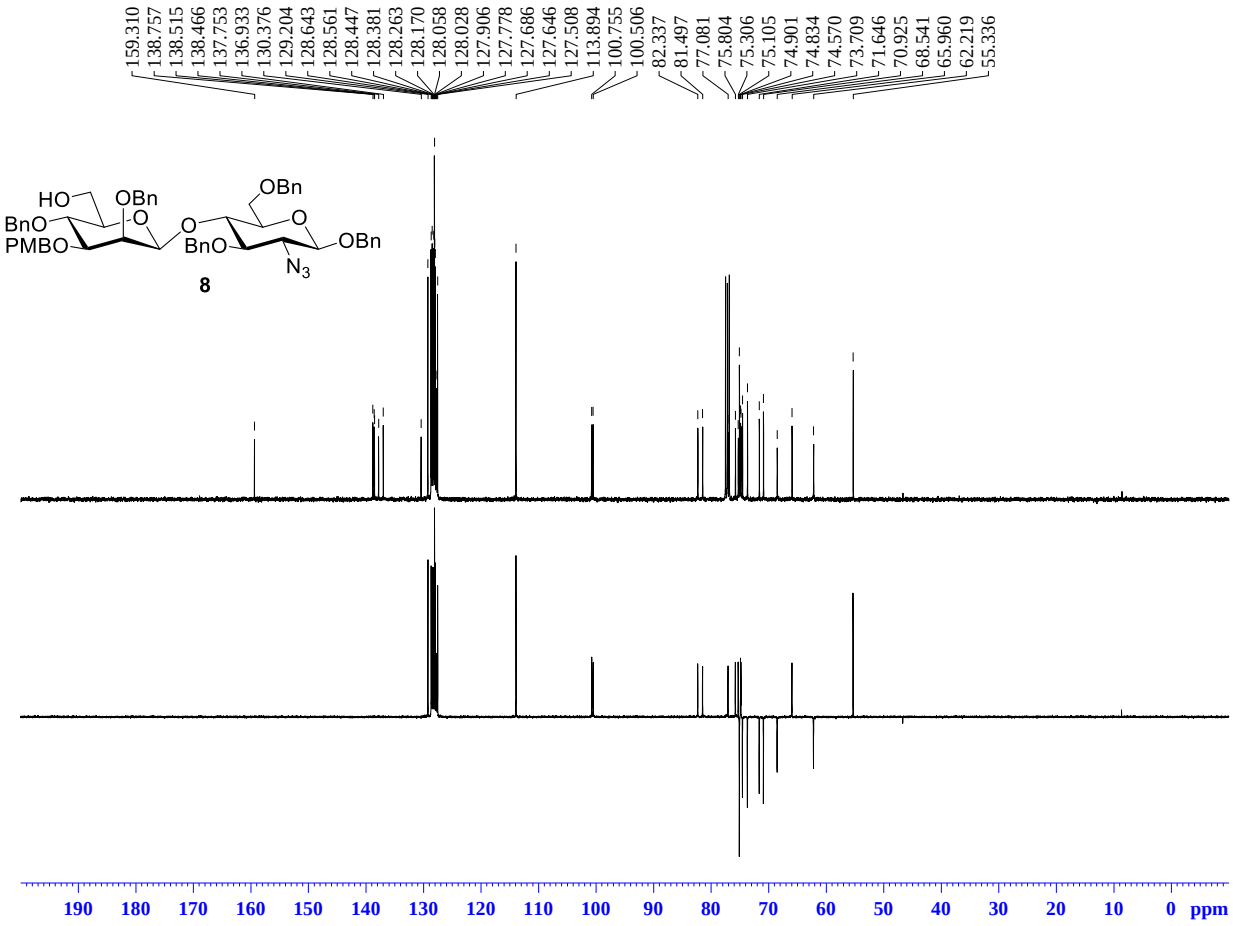
Compound 7: ^{1}H NMR (D_2O, 400 MHz)



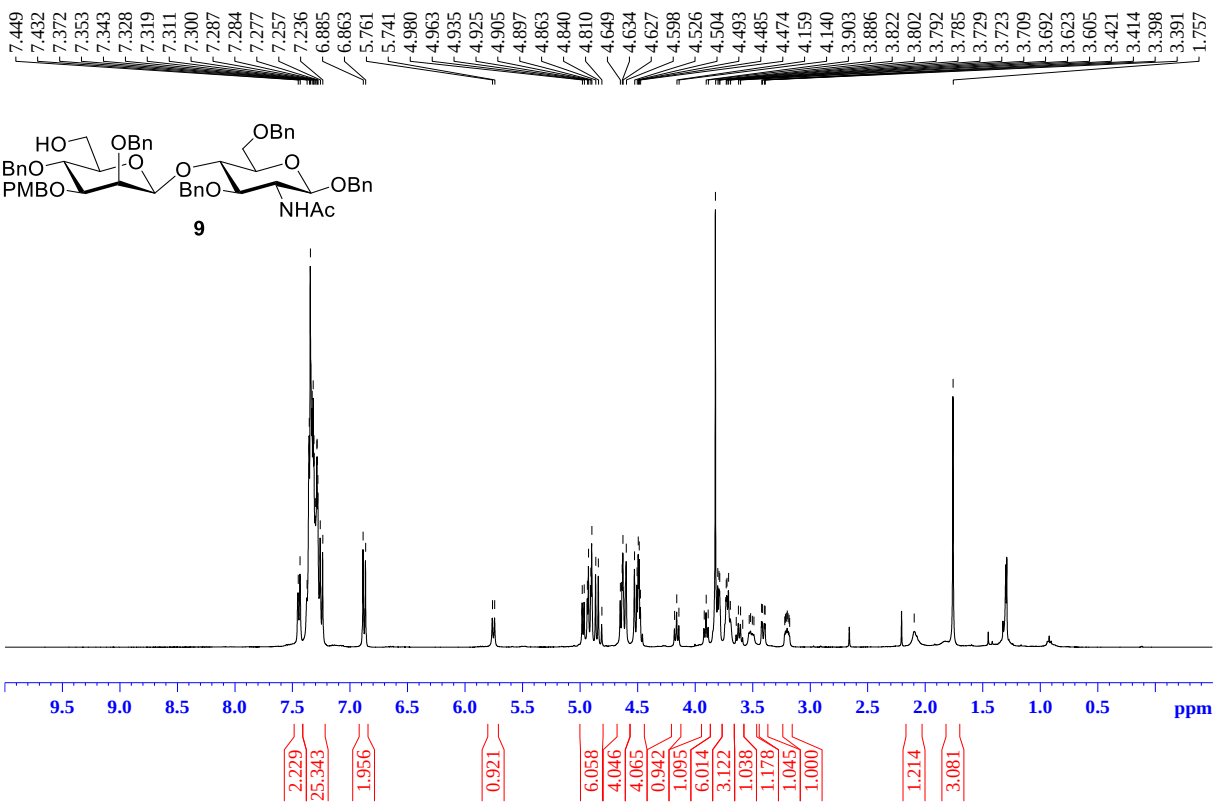
Compound 7: ^{13}C and Dept-135 NMR (D_2O, 100 MHz)



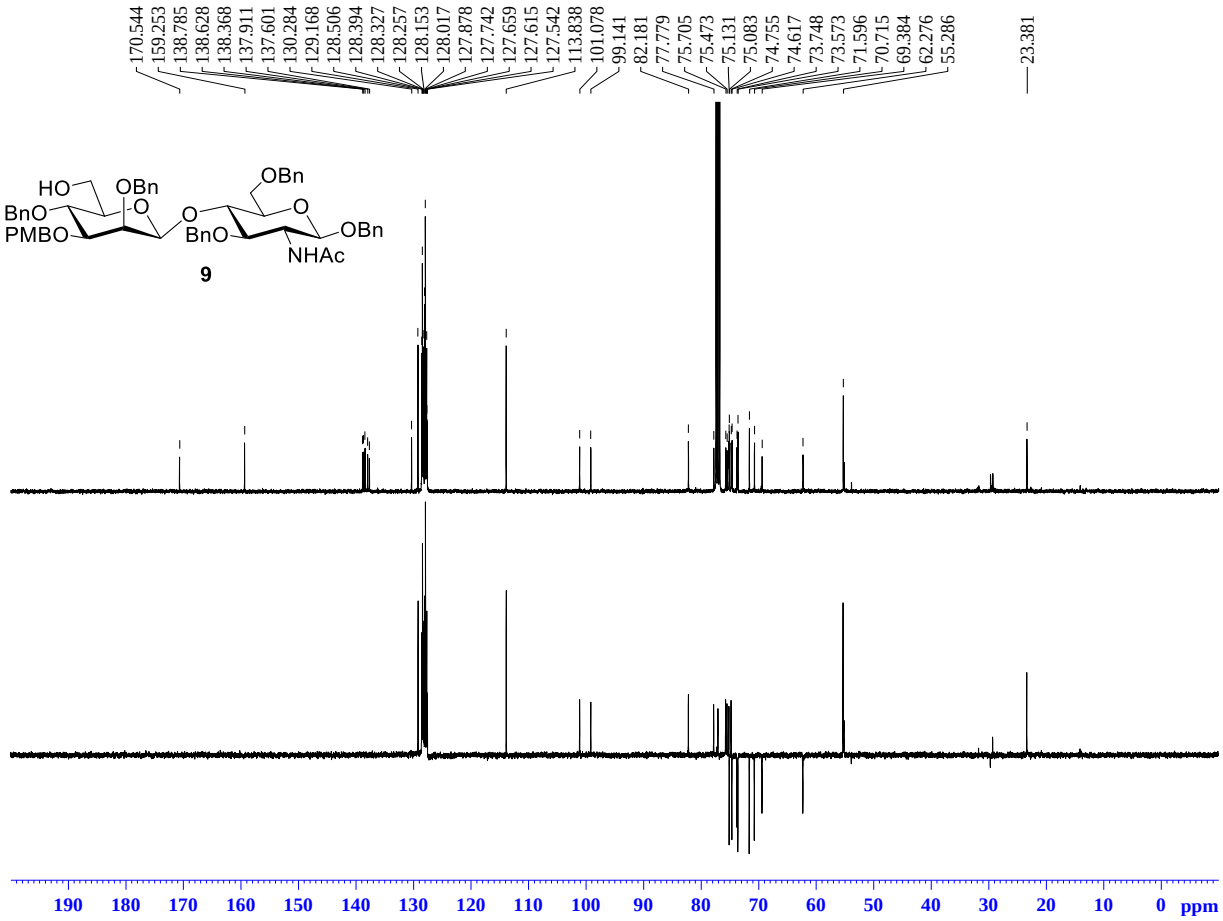
Compound 8: ^{1}H NMR ($CDCl_3$, 400 MHz)



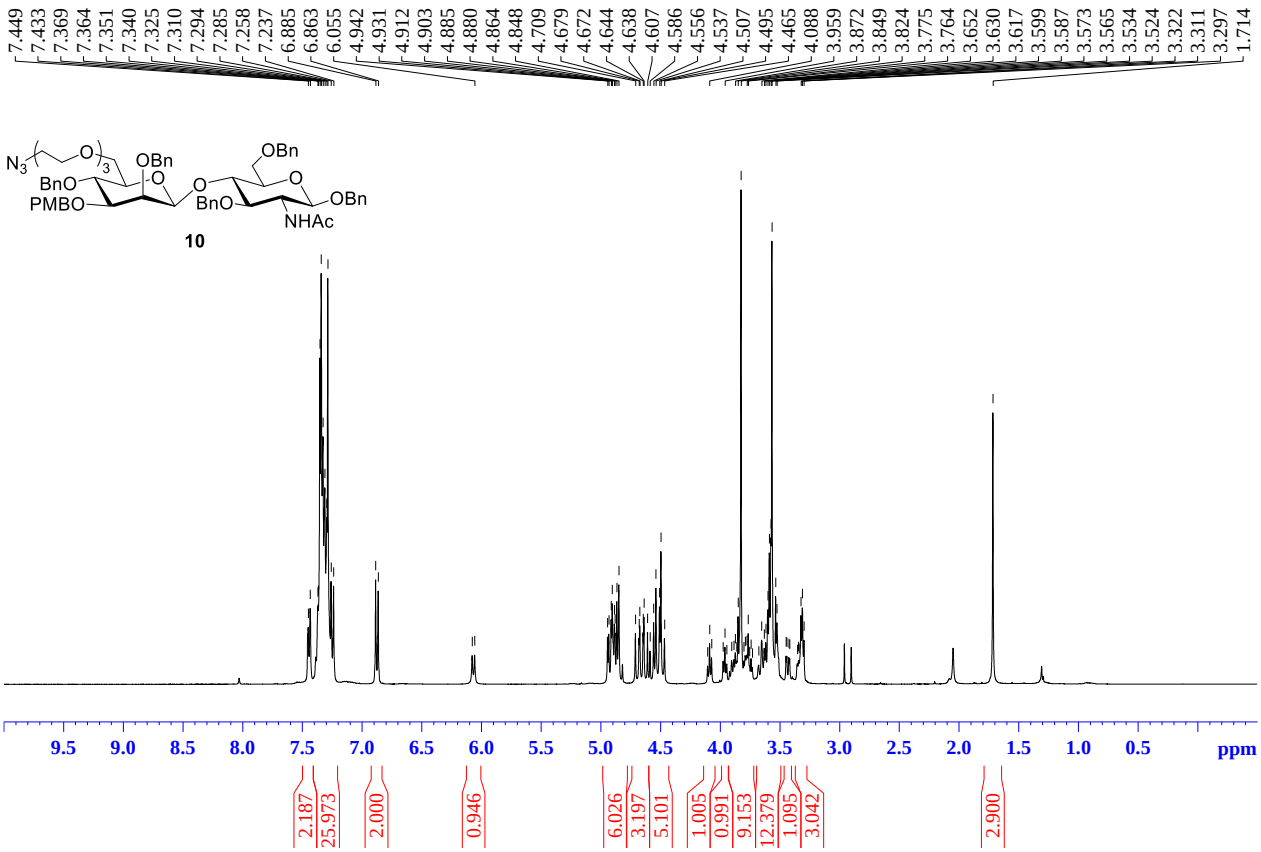
Compound 8: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



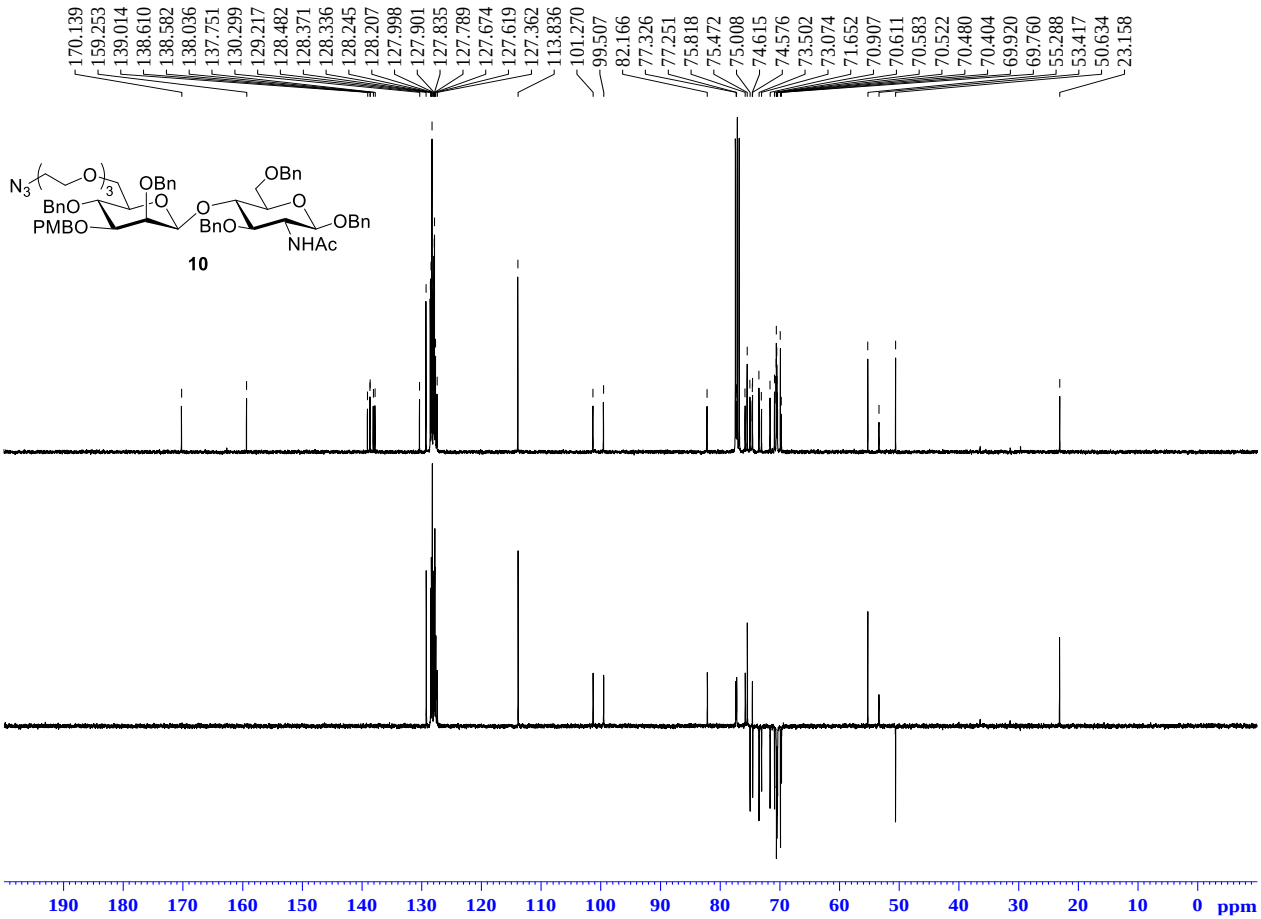
Compound 9: ^{1}H NMR ($CDCl_3$, 400 MHz)



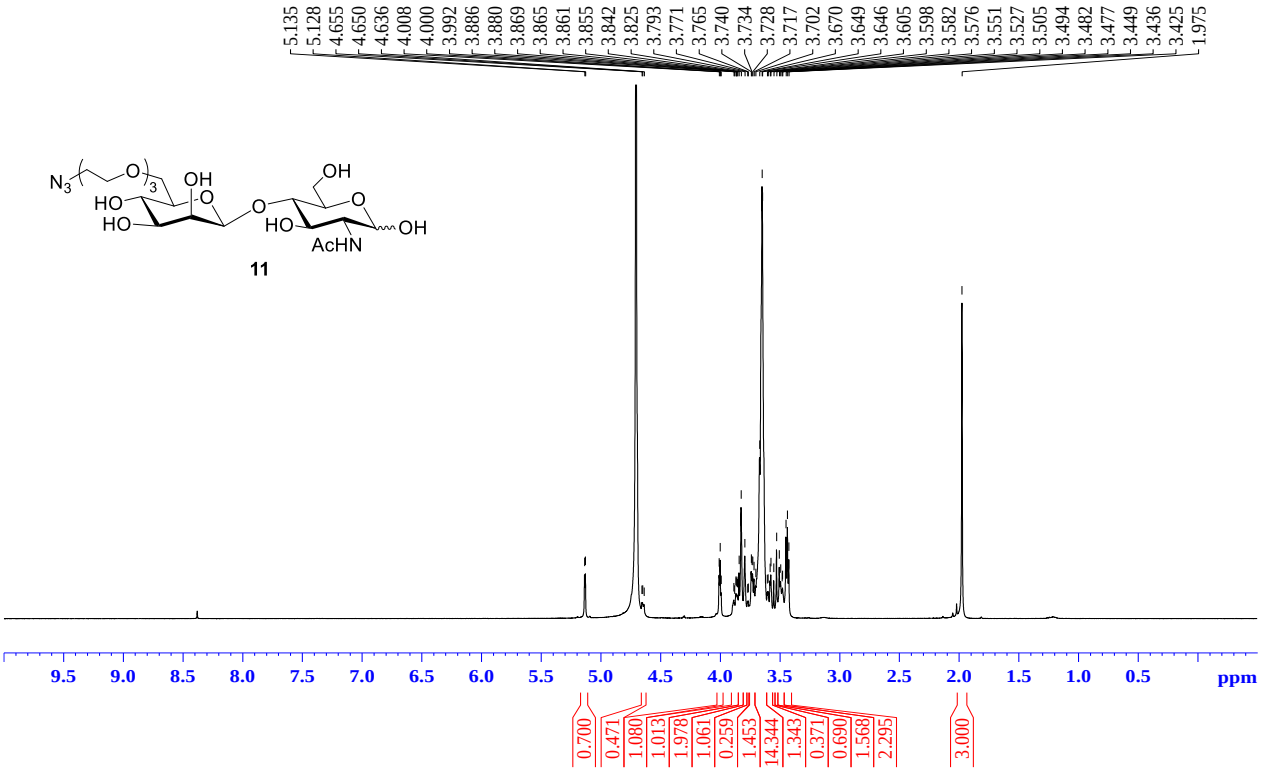
Compound 9: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



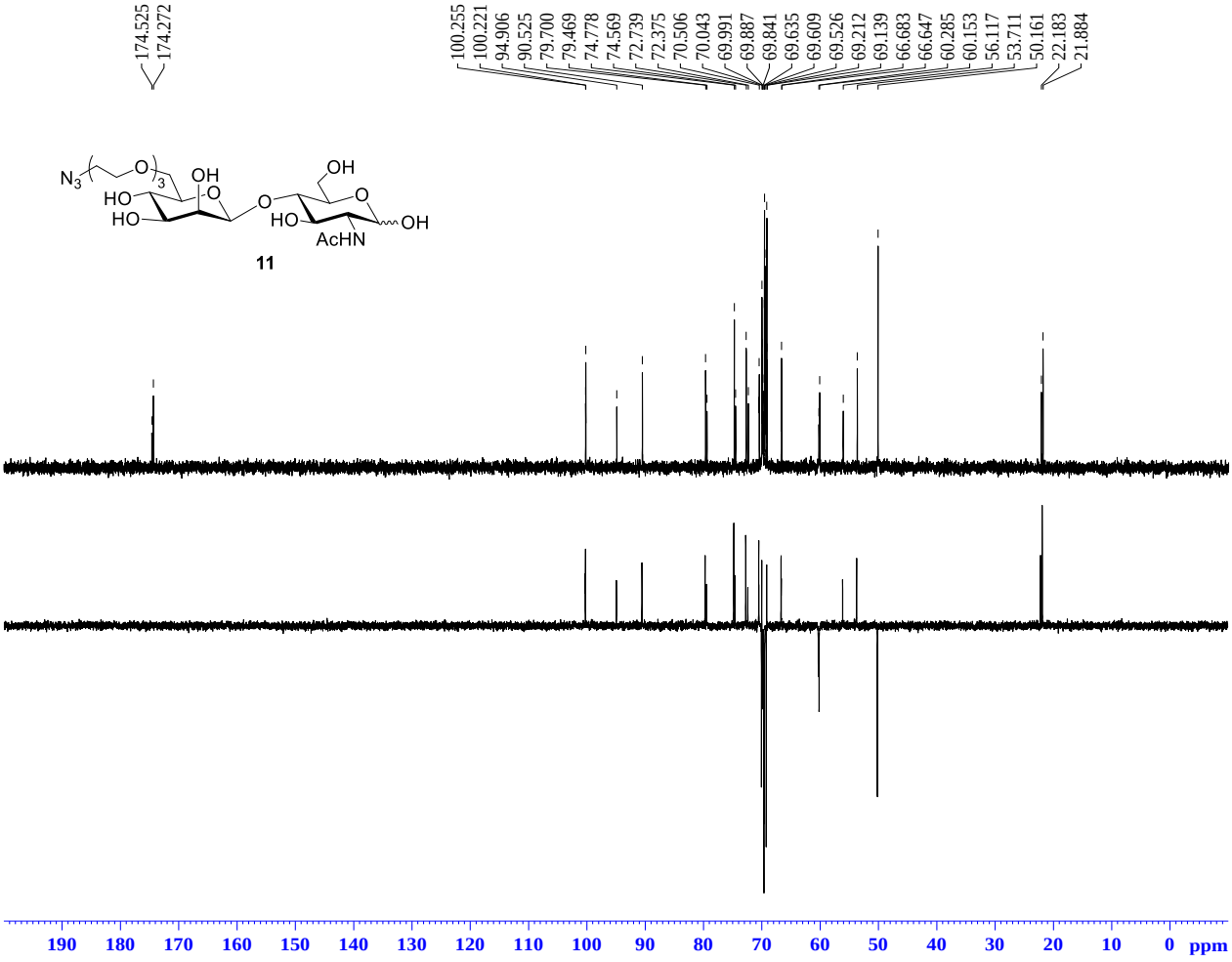
Compound 10: ^{1}H NMR ($CDCl_3$, 400 MHz)



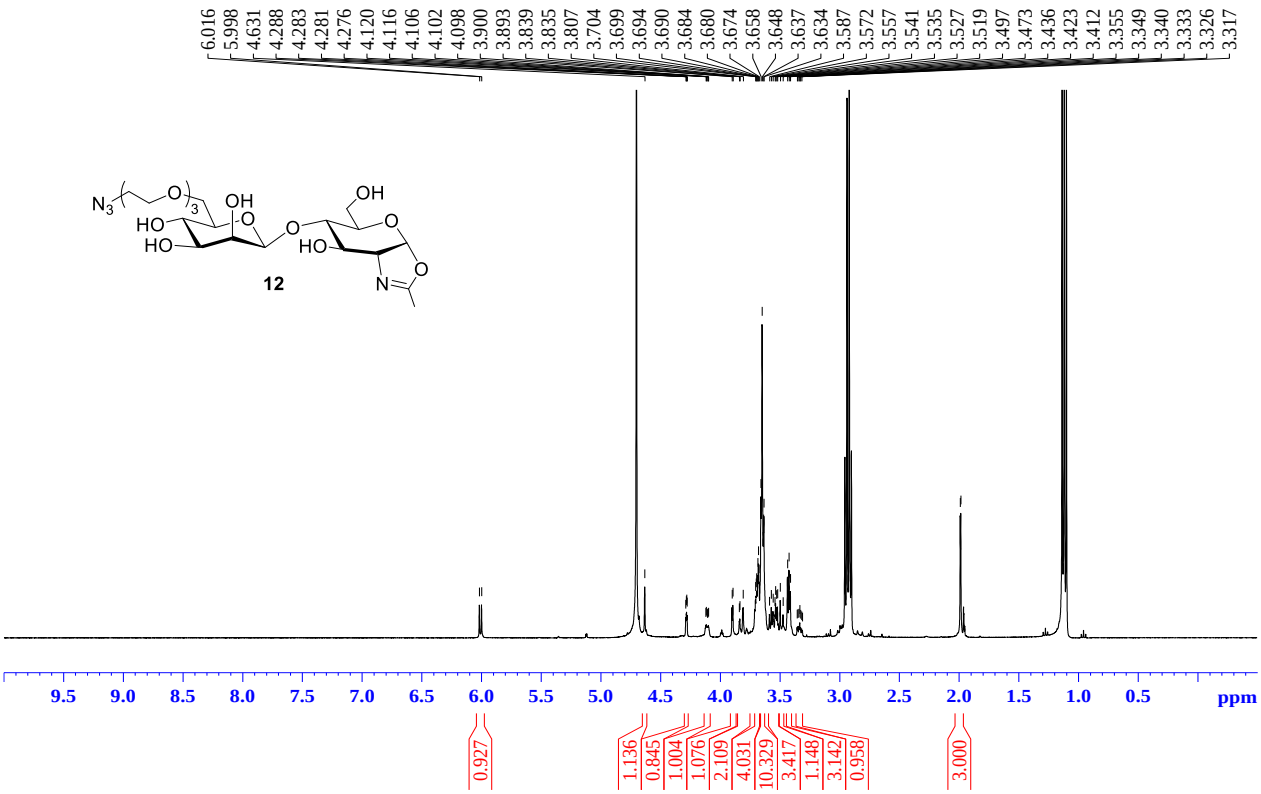
Compound 10: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



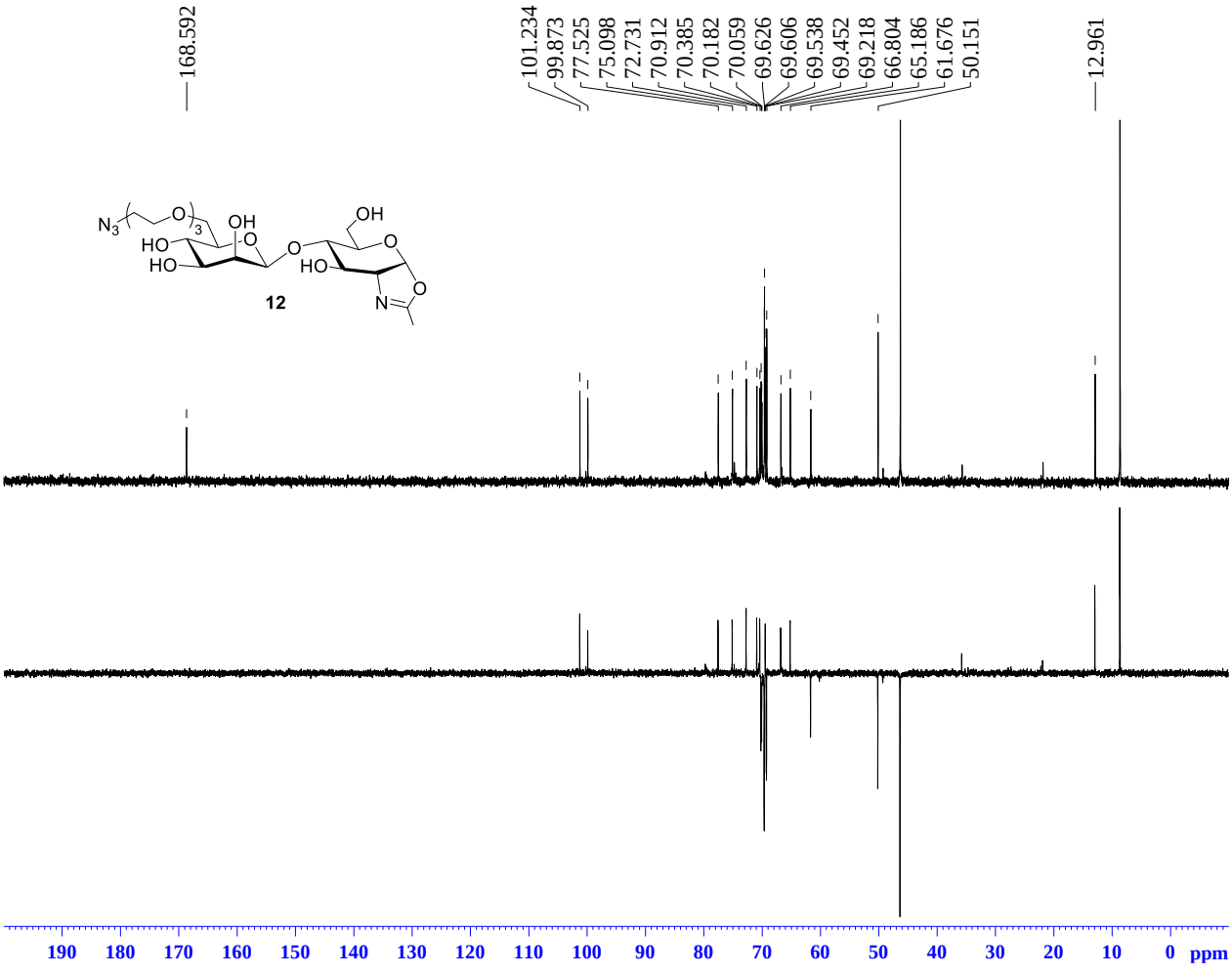
Compound 11: ^{1}H NMR (D_2O, 400 MHz)



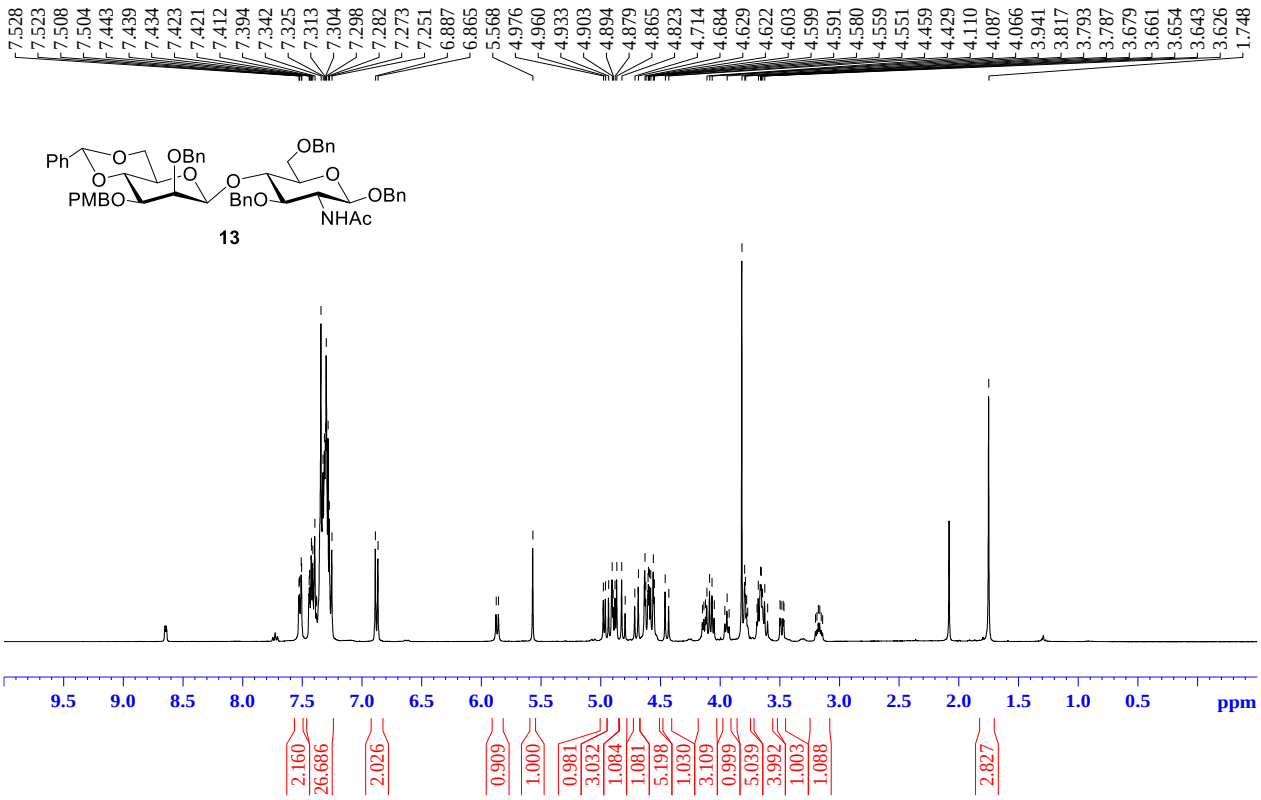
Compound 11: ^{13}C and Dept-135 NMR (D_2O, 100 MHz)



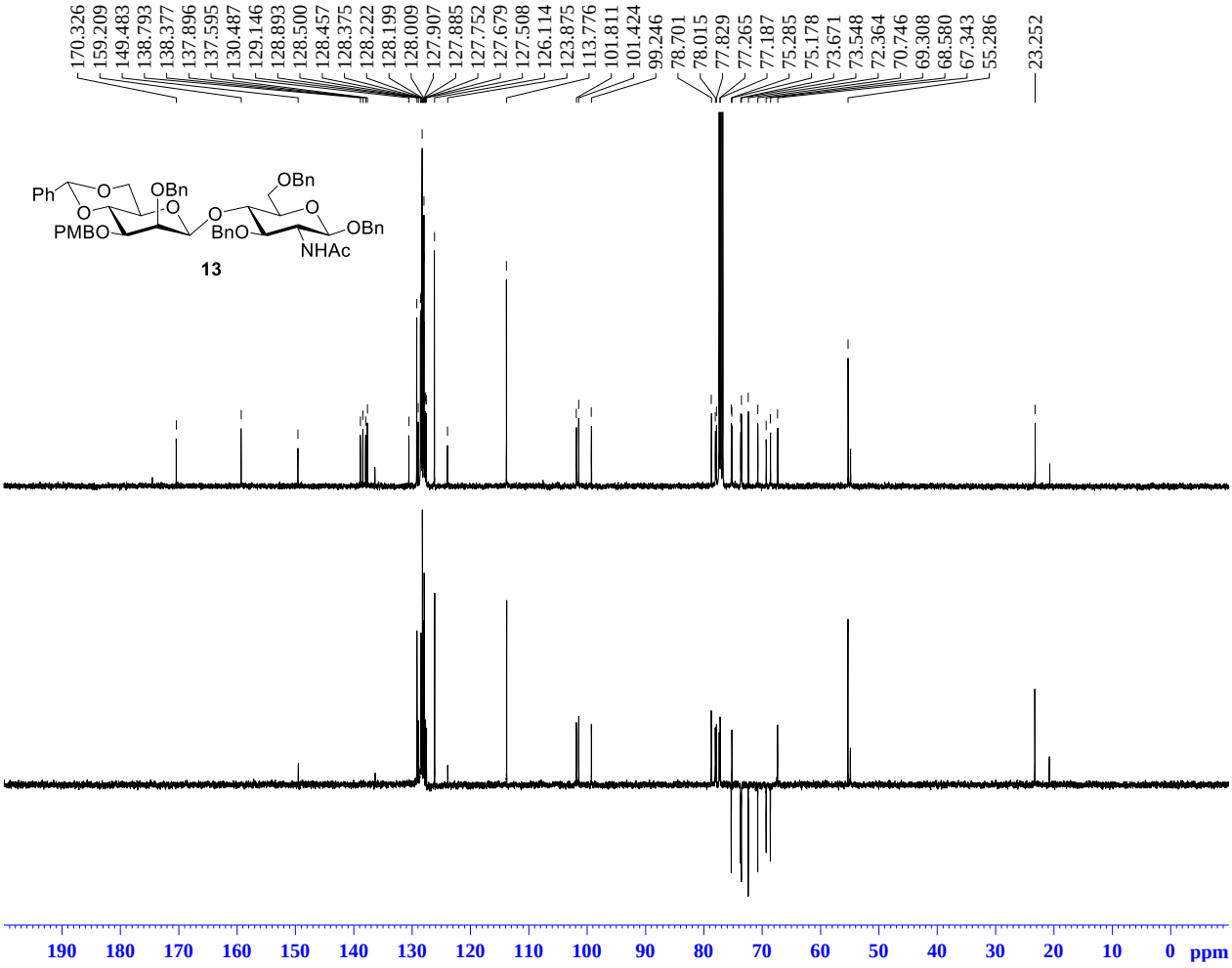
Compound 12: ^{1}H NMR (D_2O, 400 MHz)



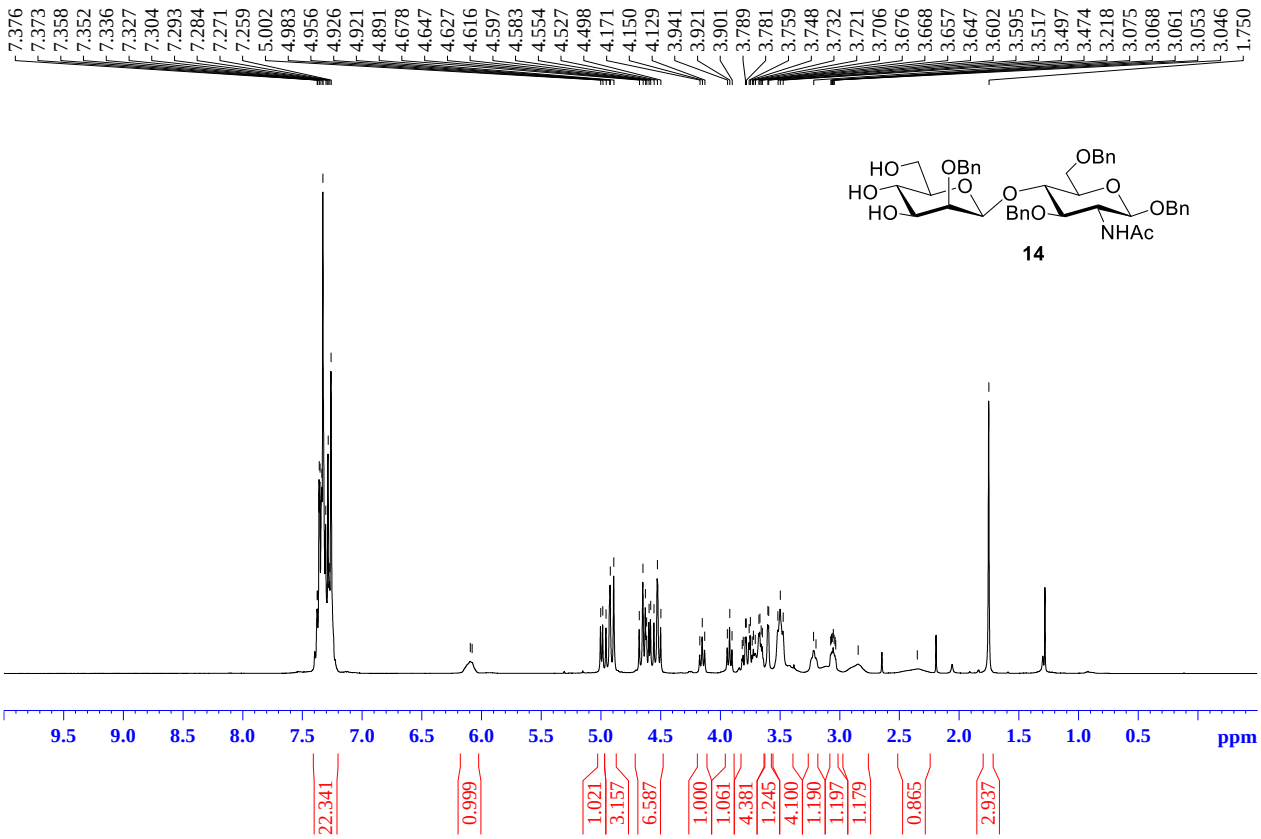
Compound 12: ^{13}C and Dept-135 NMR (D_2O, 100 MHz)



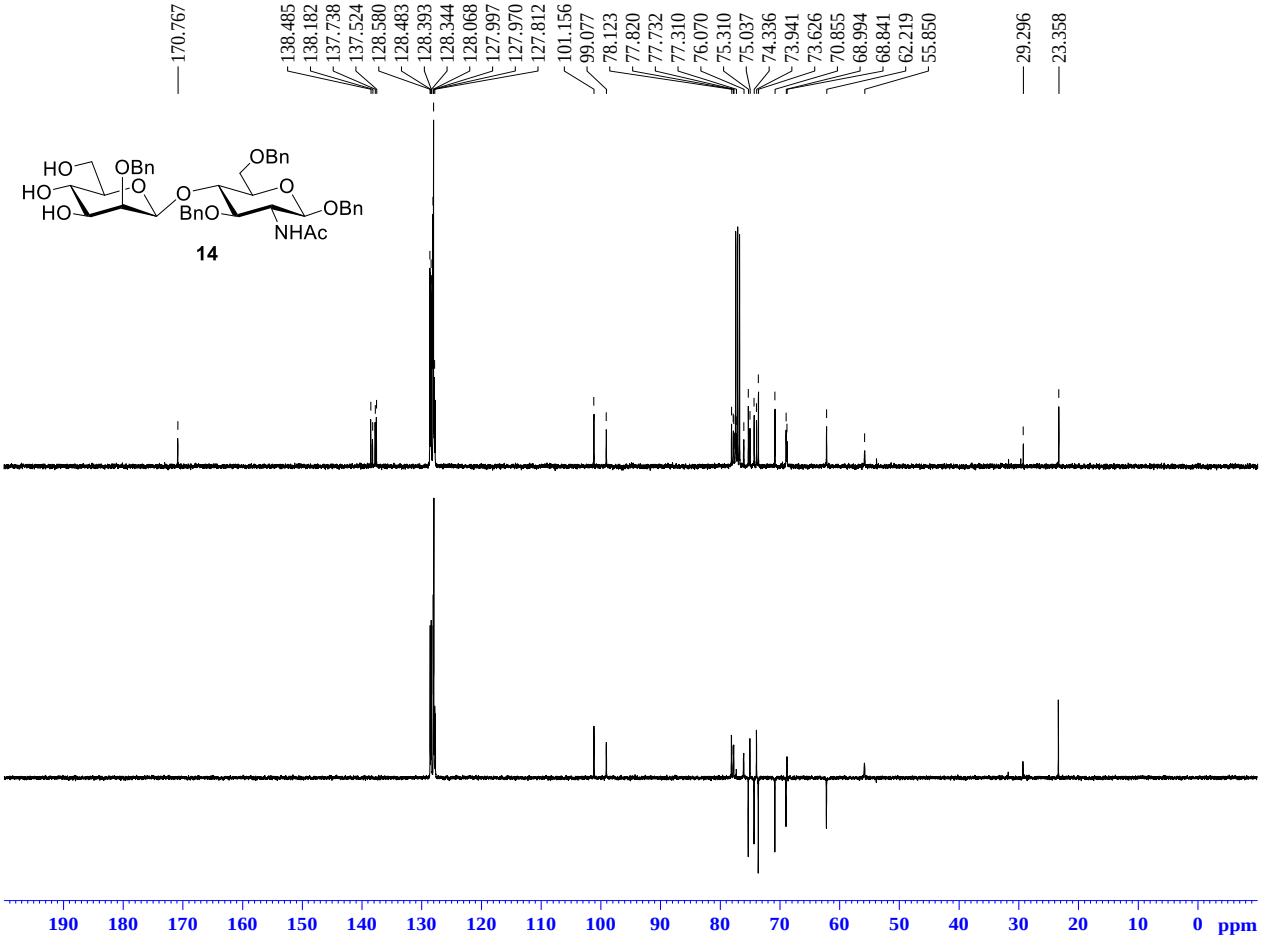
Compound 13: ^{1}H NMR ($CDCl_3$, 400 MHz)



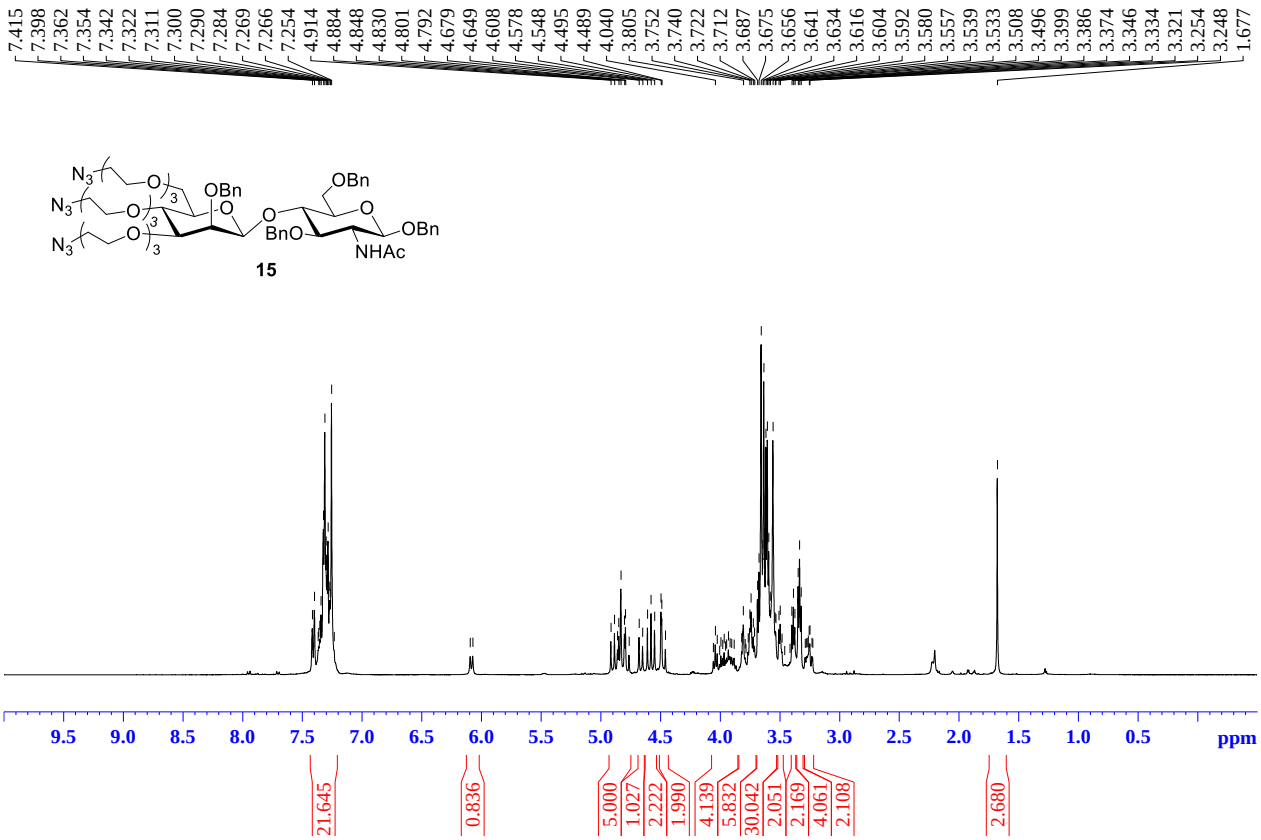
Compound 13: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



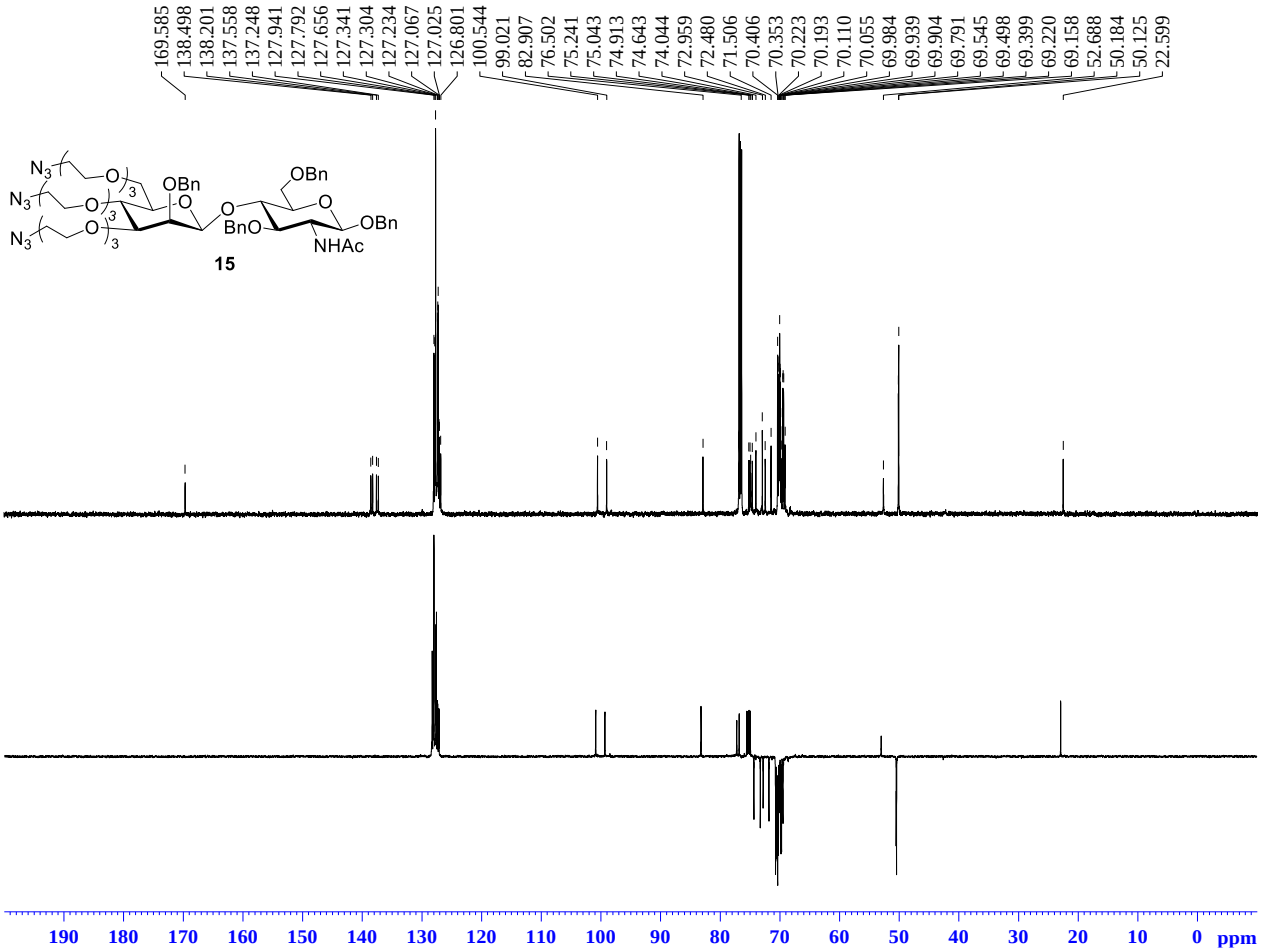
Compound 14: ^{1}H NMR ($CDCl_3$, 400 MHz)



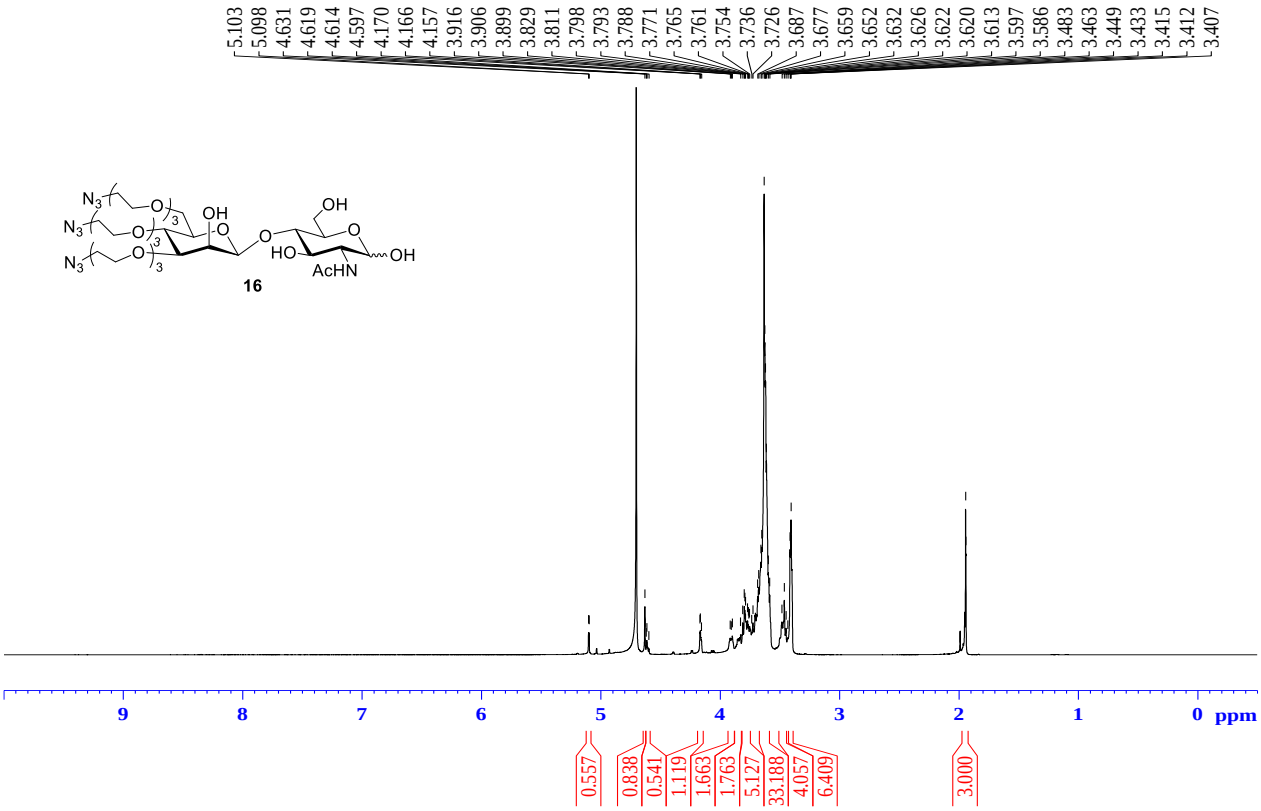
Compound 14: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



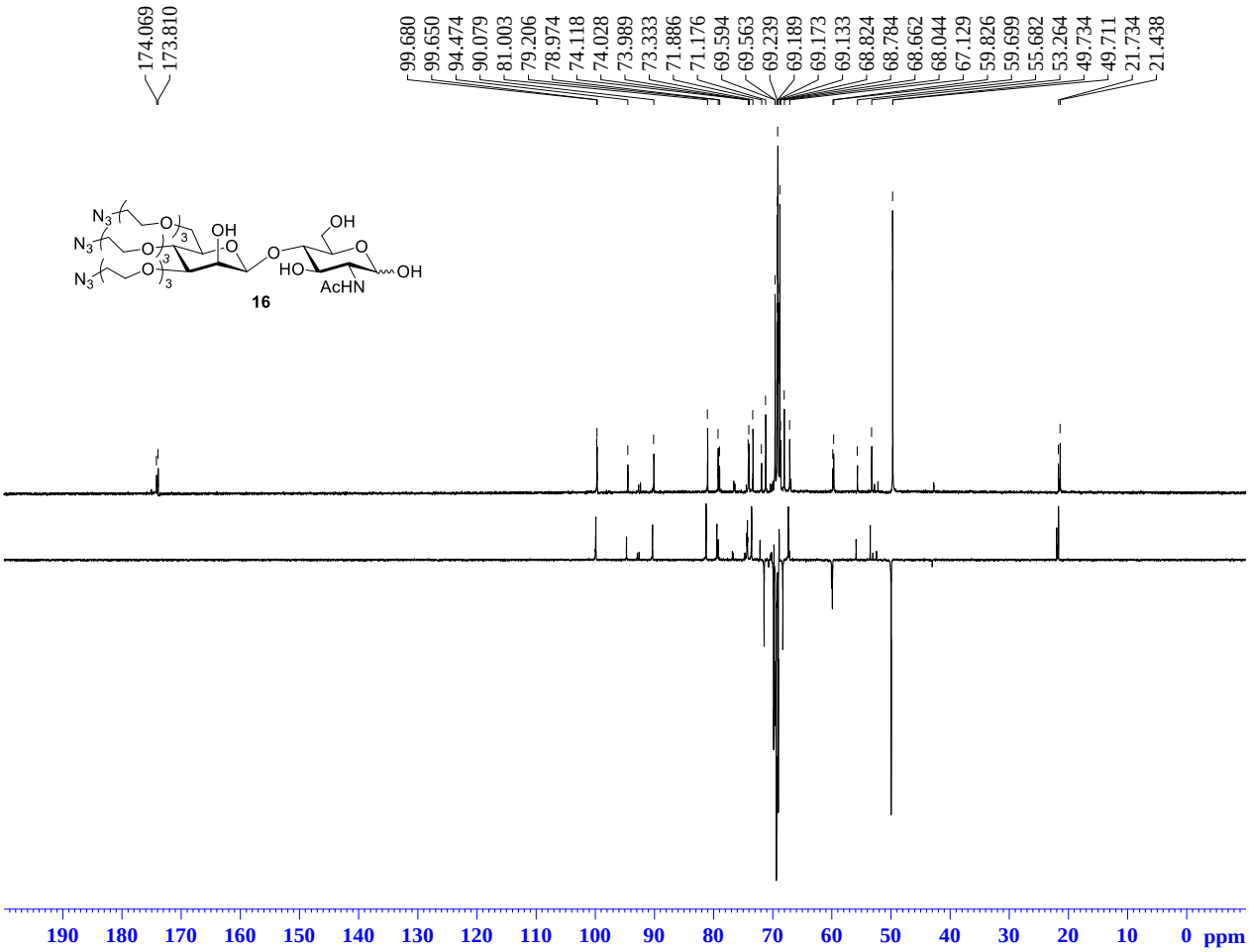
Compound 15: ^{1}H NMR ($CDCl_3$, 400 MHz)



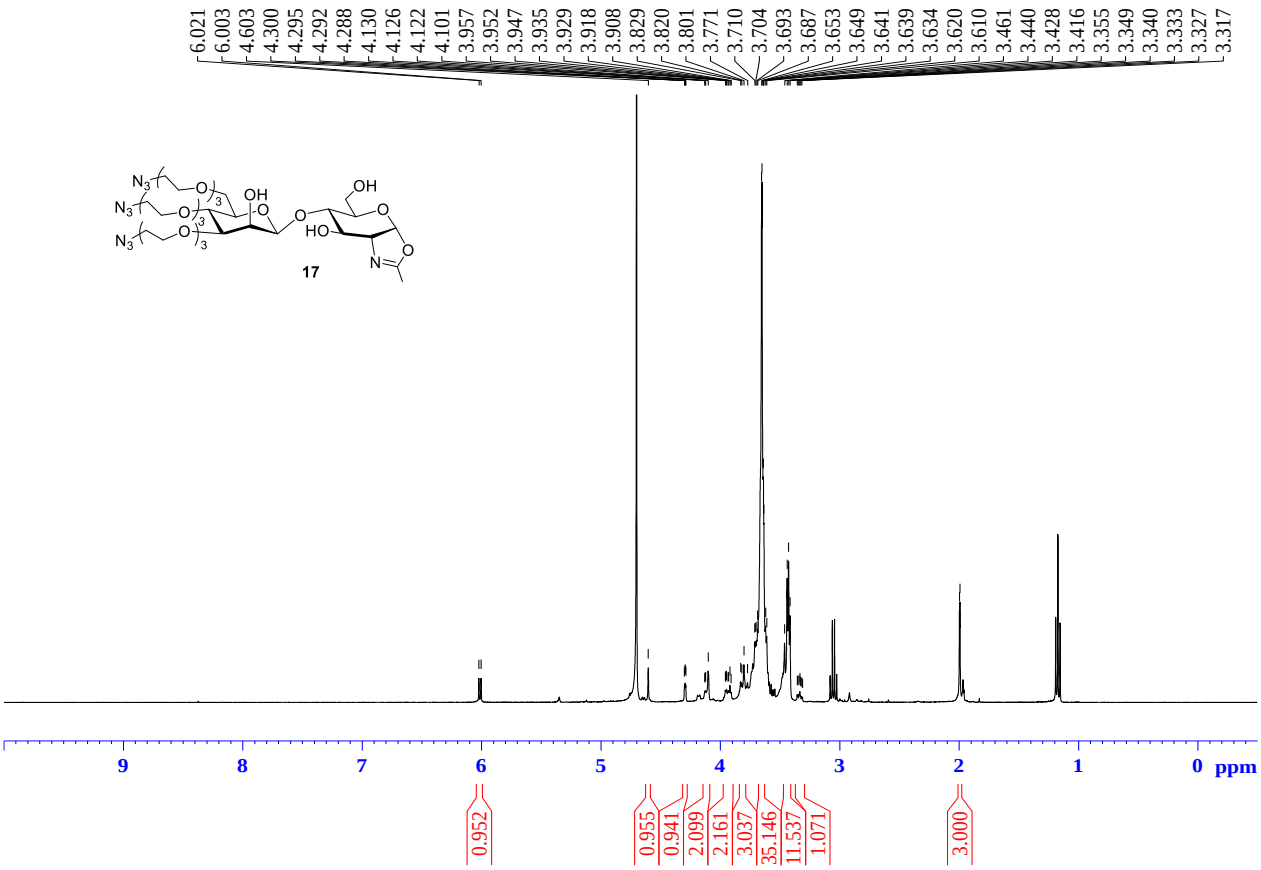
Compound 15: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



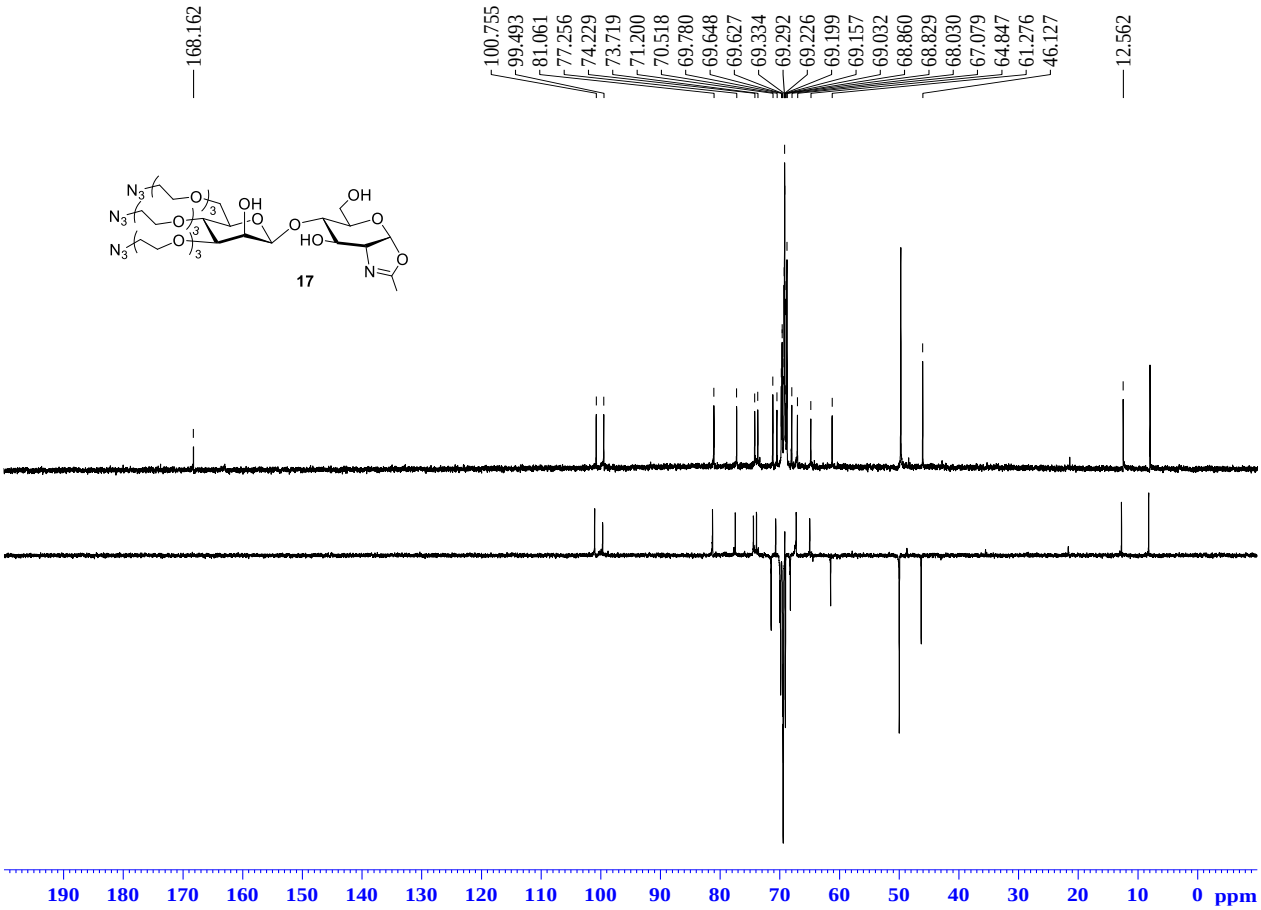
Compound 16: ^{1}H NMR (D_2O, 400 MHz)



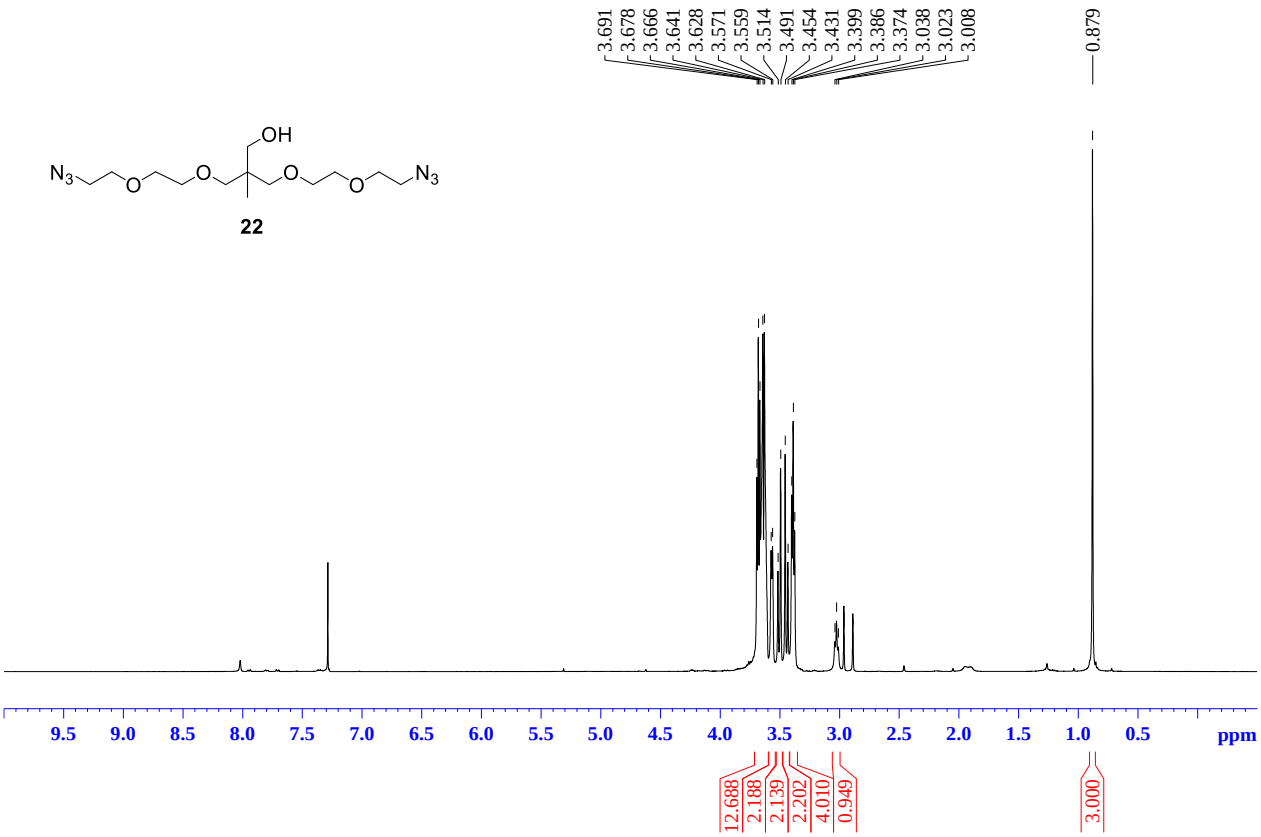
Compound 16: ^{13}C and Dept-135 NMR (D_2O, 100 MHz)



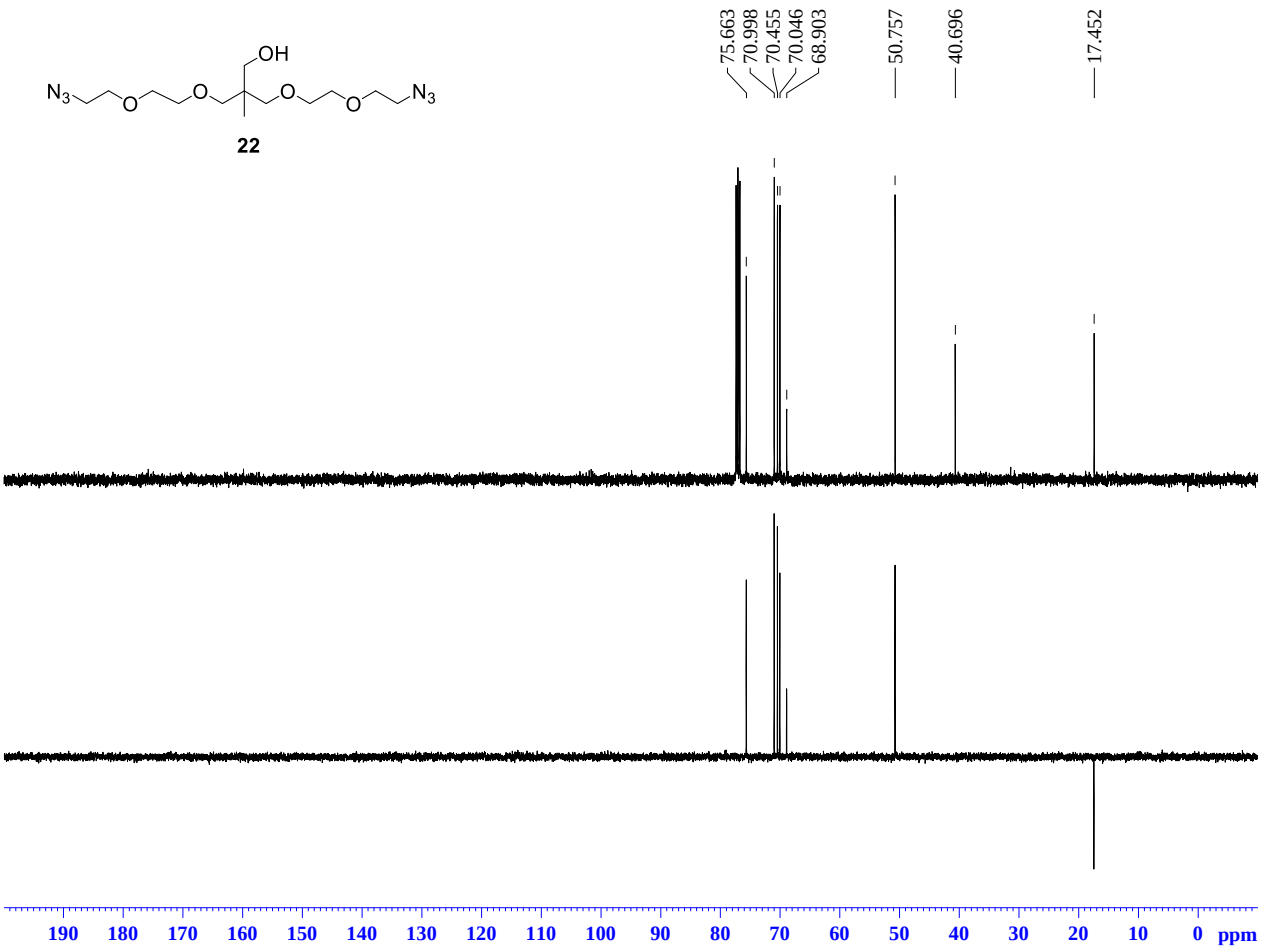
Compound 17: ^{1}H NMR (D_2O, 400 MHz)



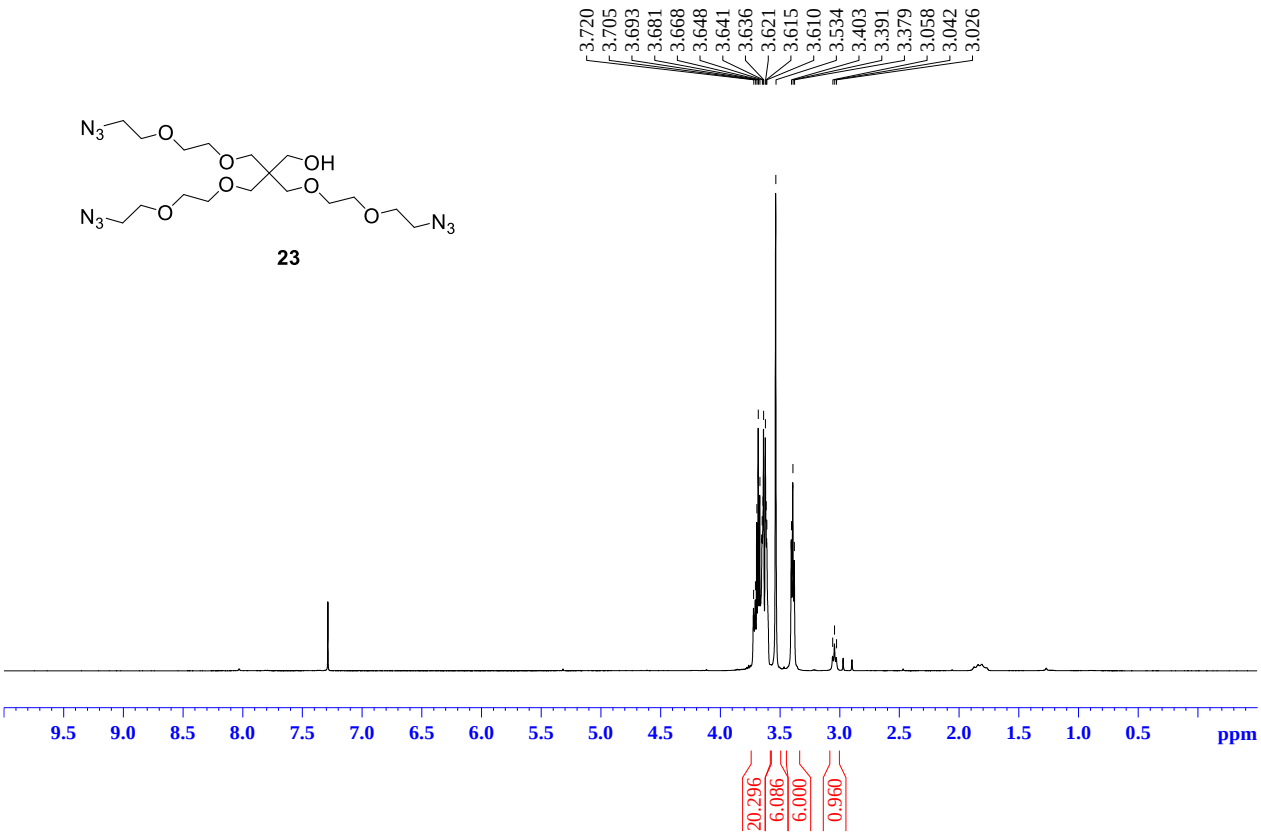
Compound 17: ^{13}C and Dept-135 NMR (D_2O, 100 MHz)



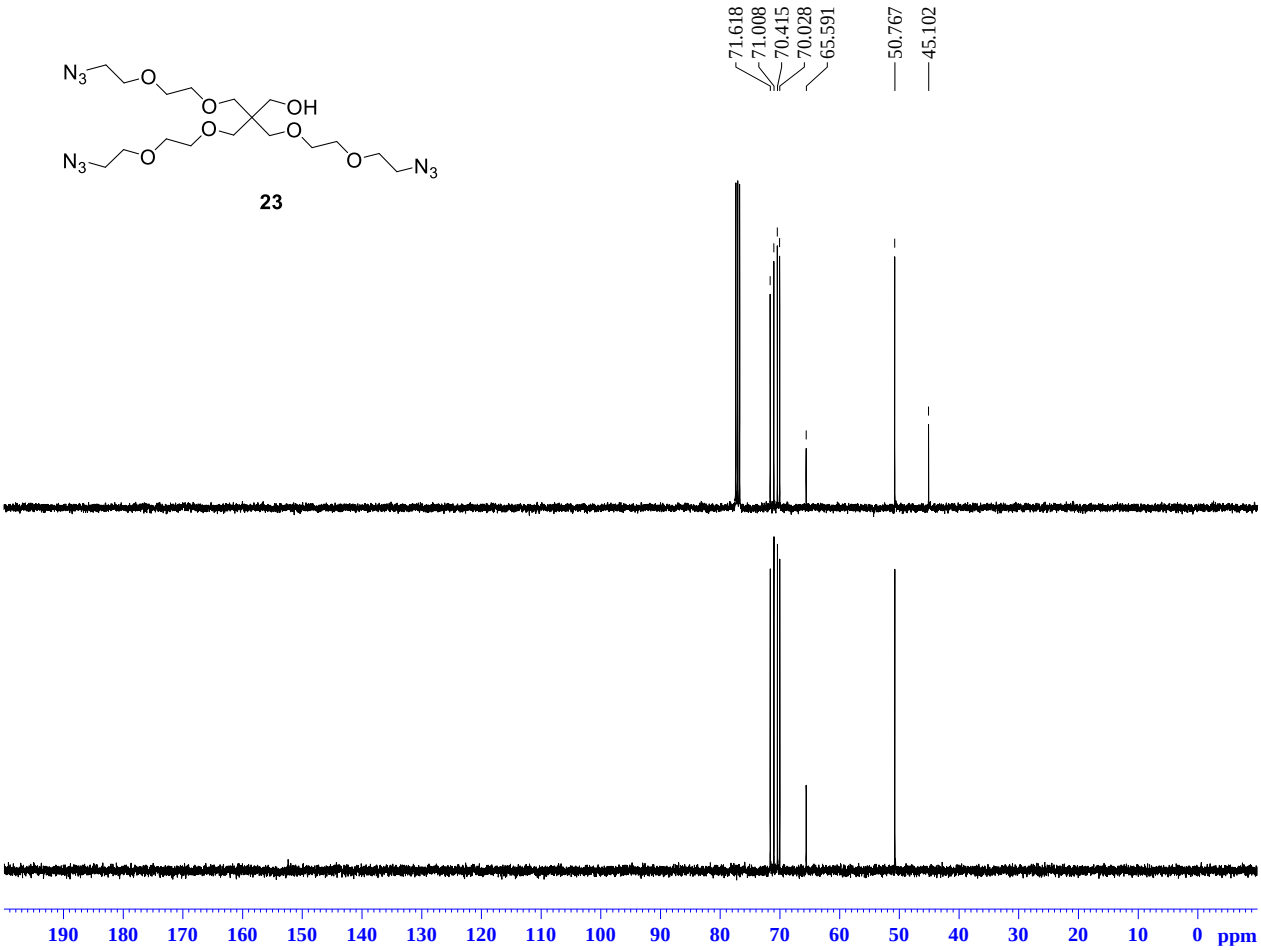
Compound 22: ^{1}H NMR ($CDCl_3$, 400 MHz)



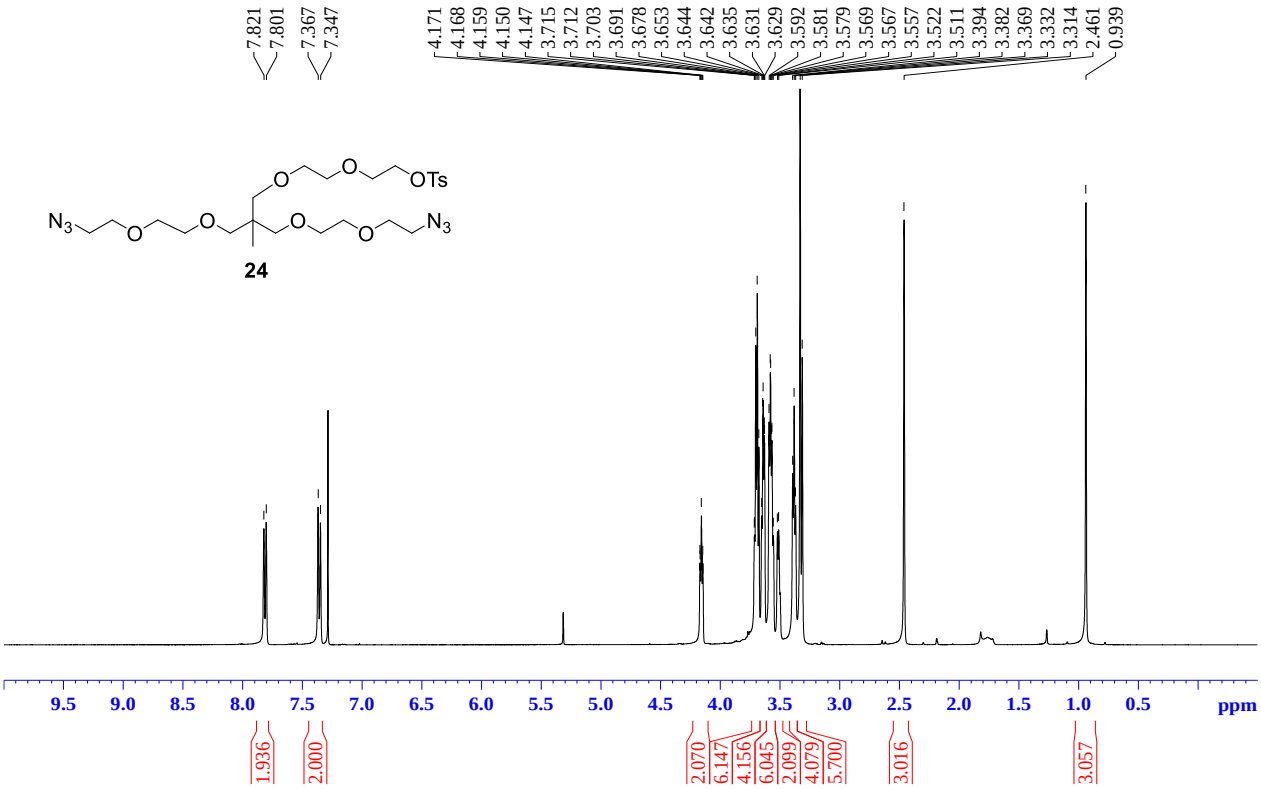
Compound 22: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



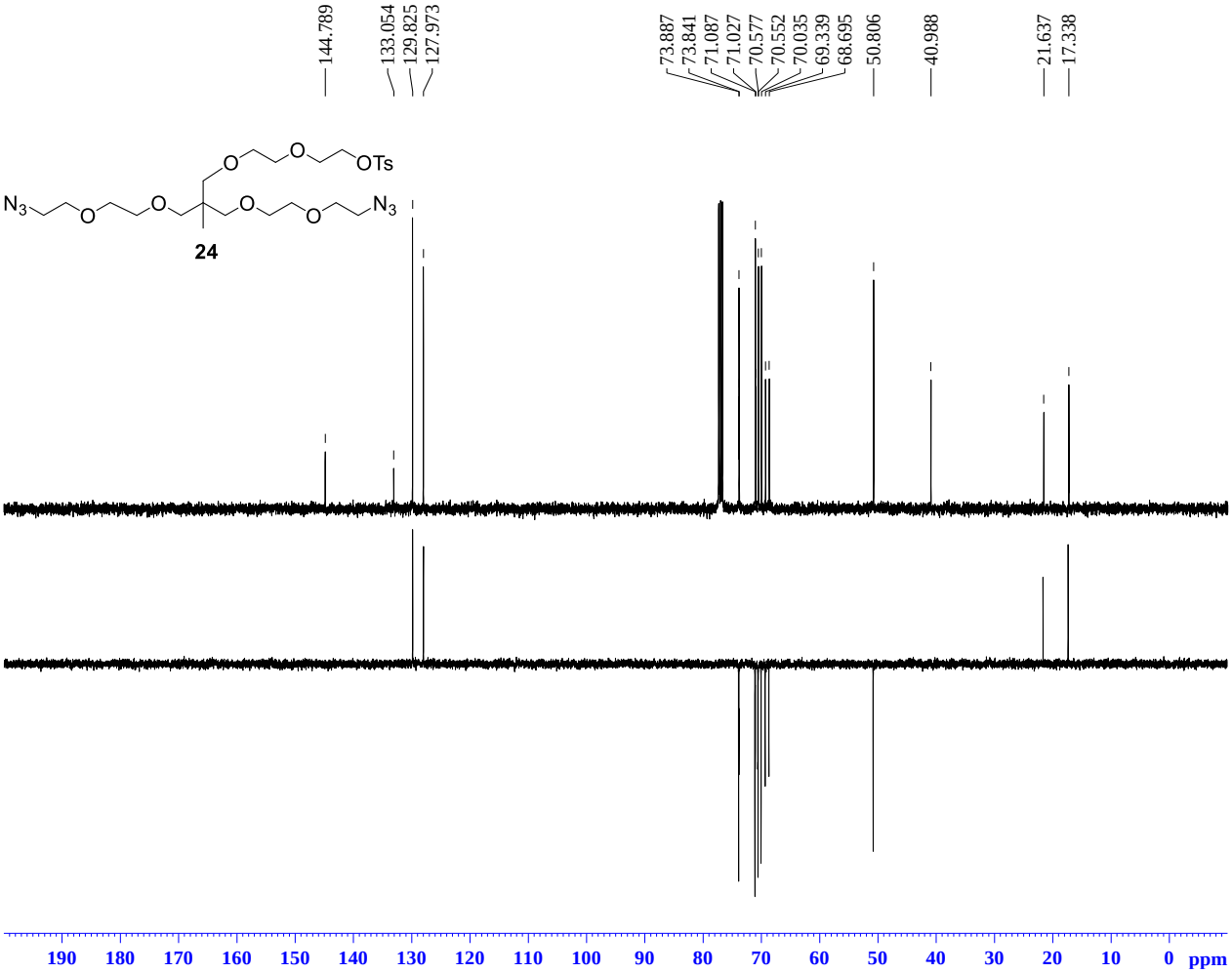
Compound 23: ^{1}H NMR ($CDCl_3$, 400 MHz)



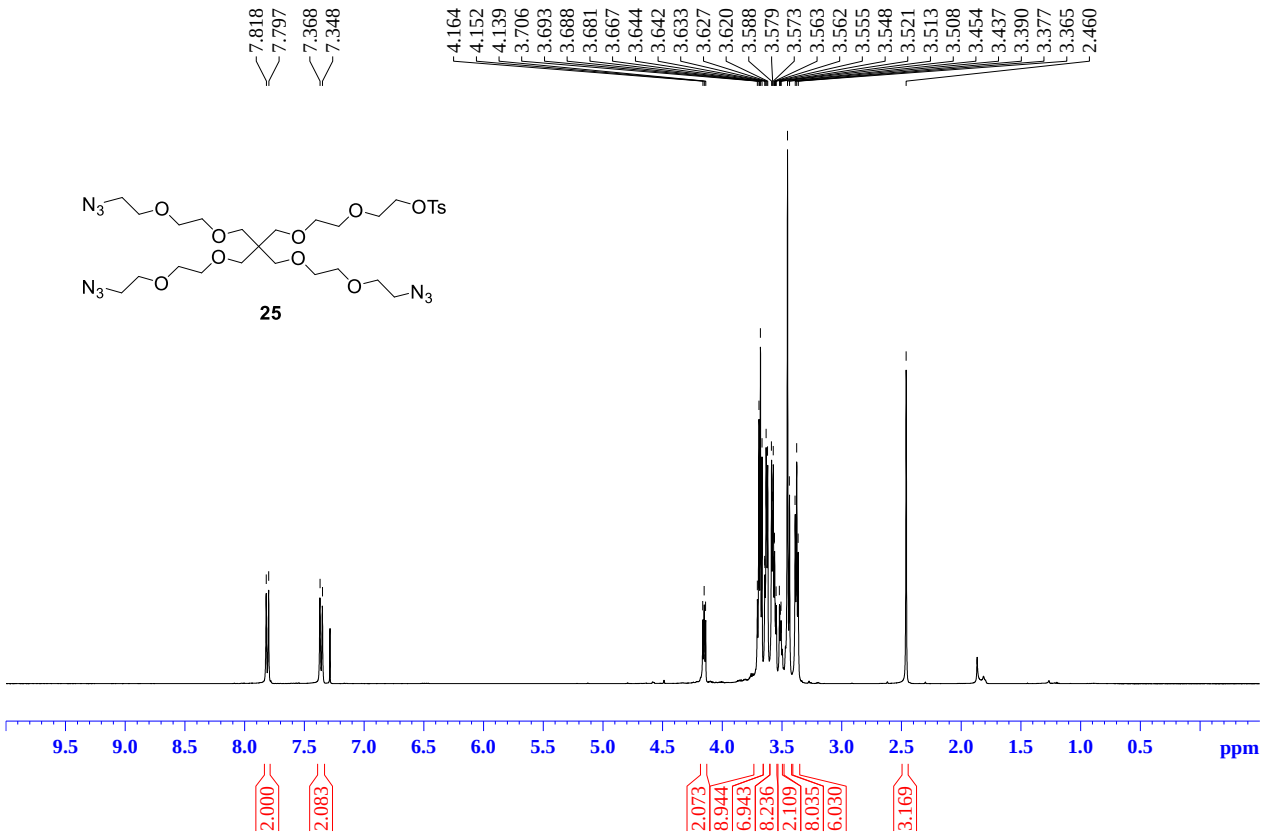
Compound 23: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



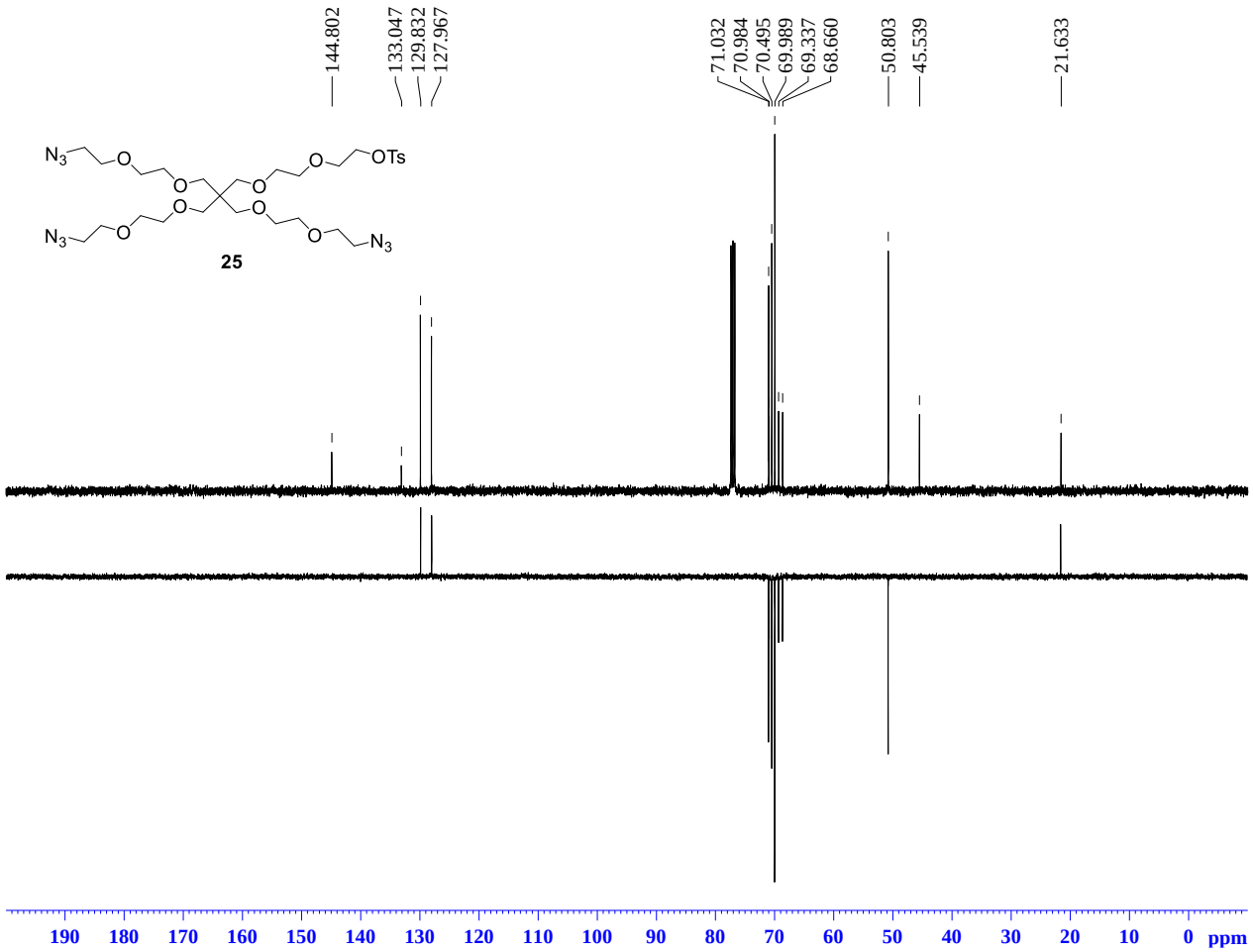
Compound 24: ^{1}H NMR ($CDCl_3$, 400 MHz)



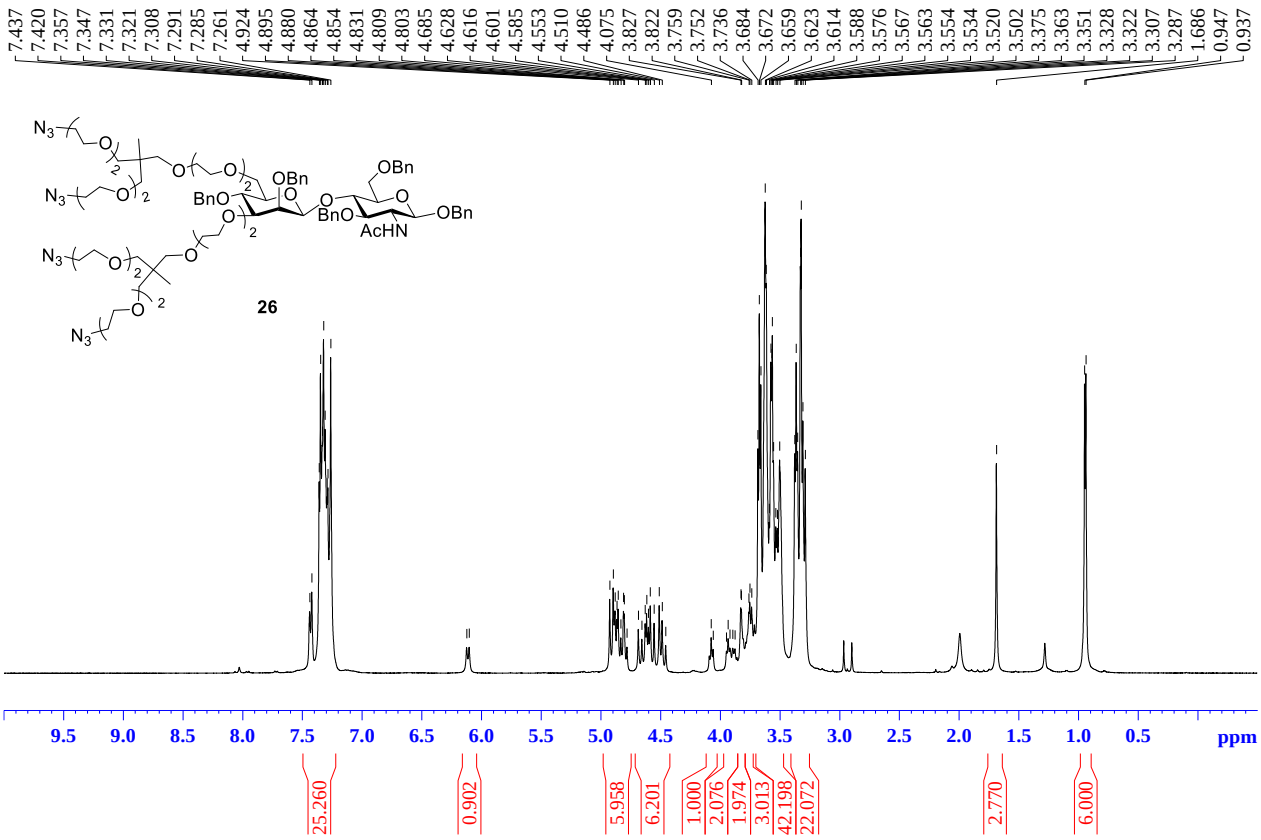
Compound 24: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



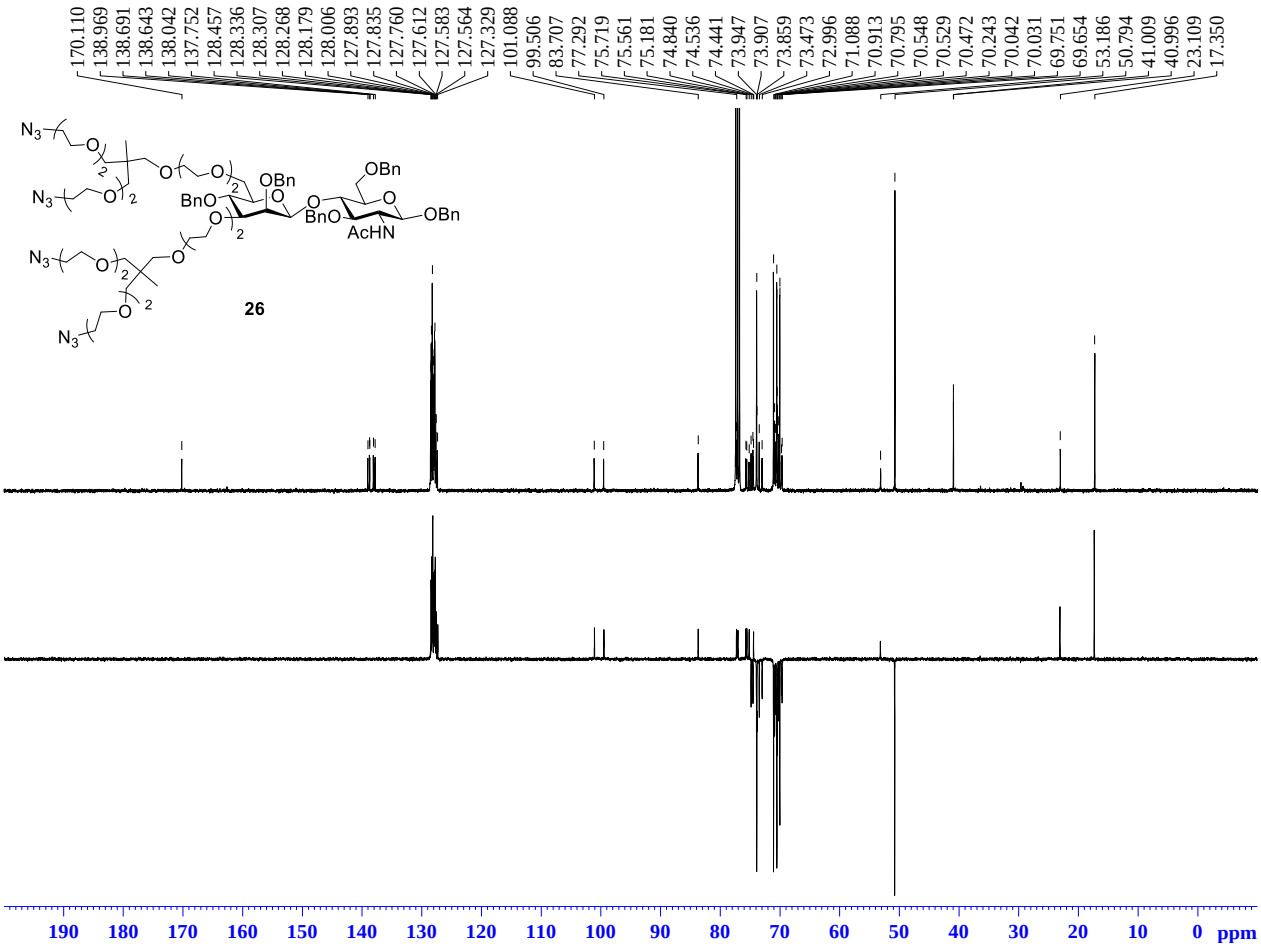
Compound 25: ^{1}H NMR ($CDCl_3$, 400 MHz)



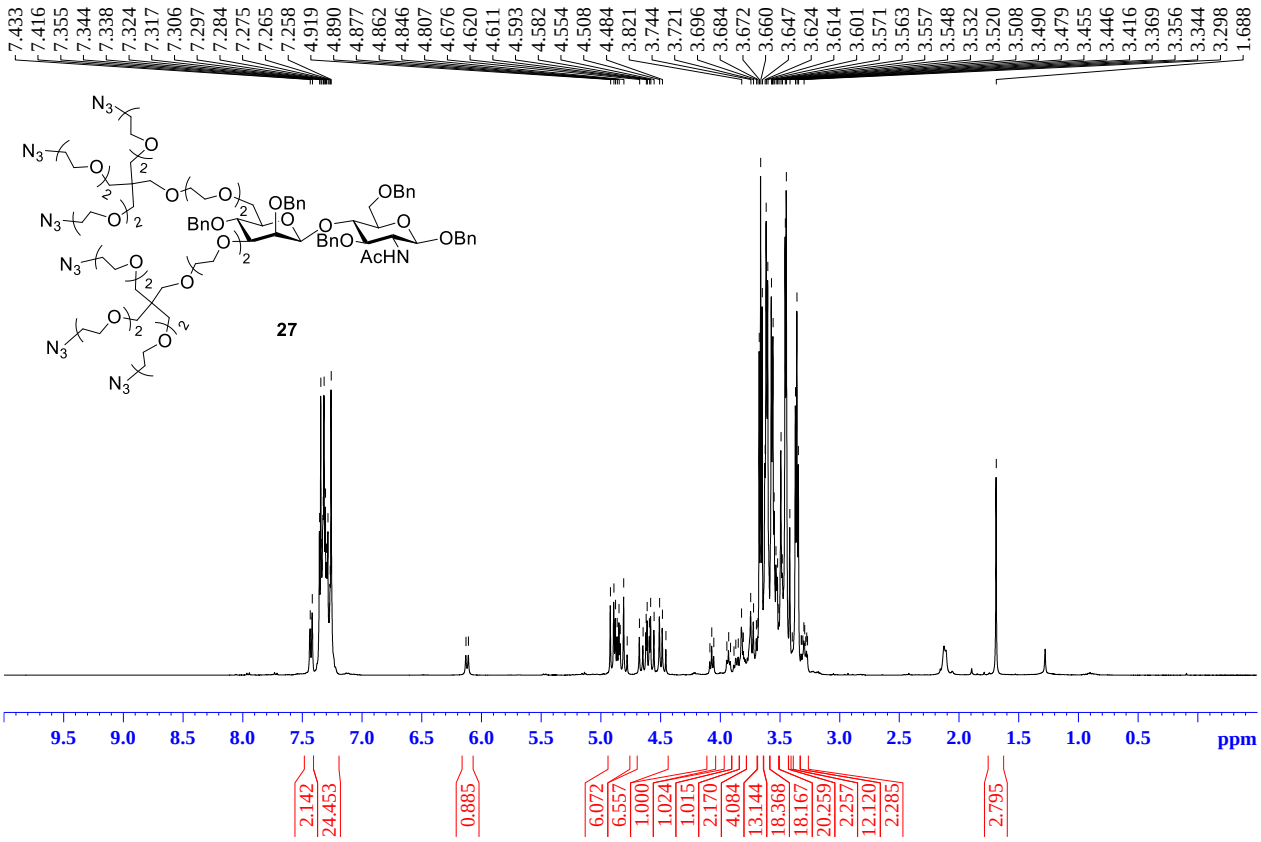
Compound 25: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



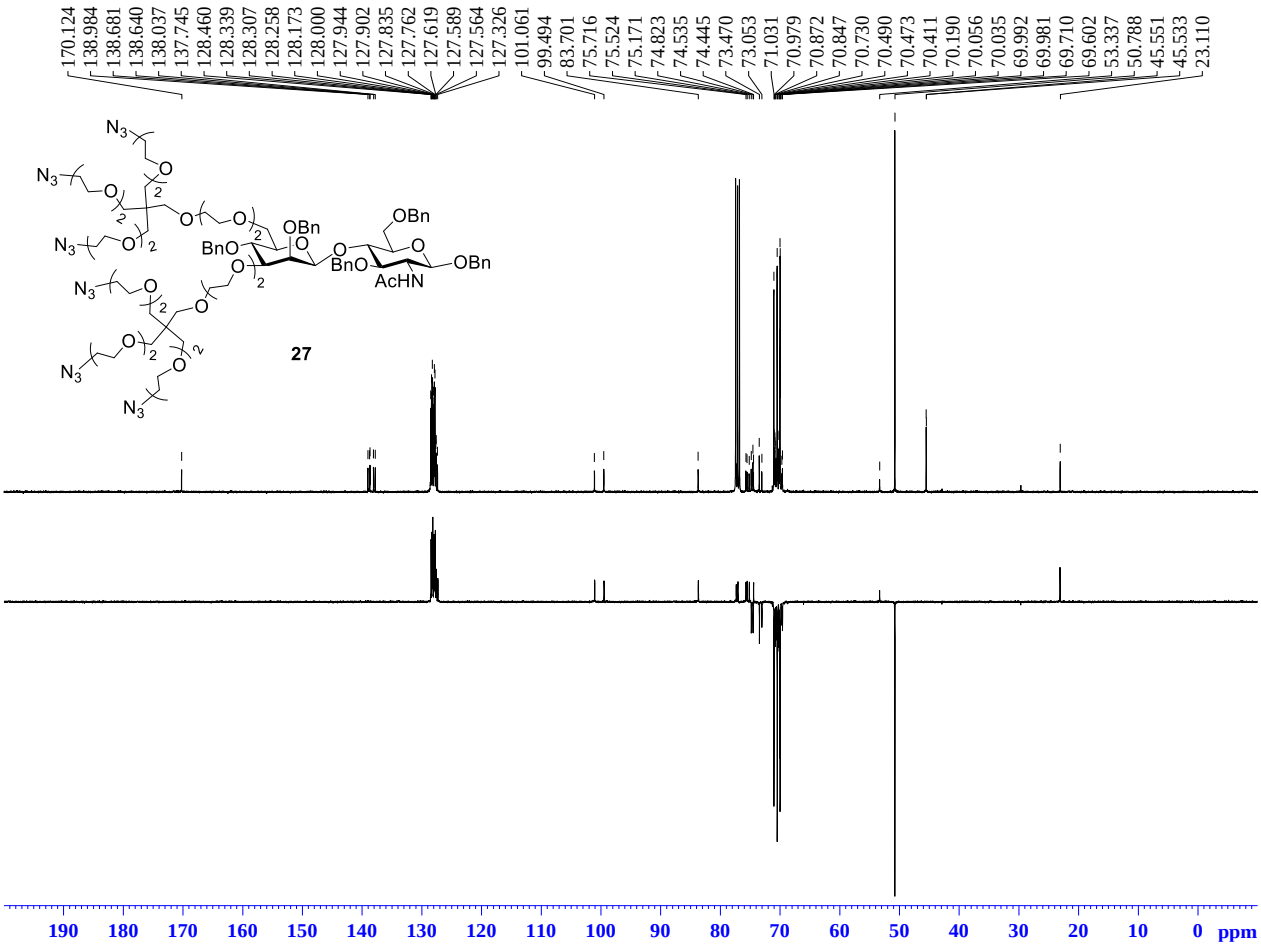
Compound 26: ^{1}H NMR ($CDCl_3$, 400 MHz)



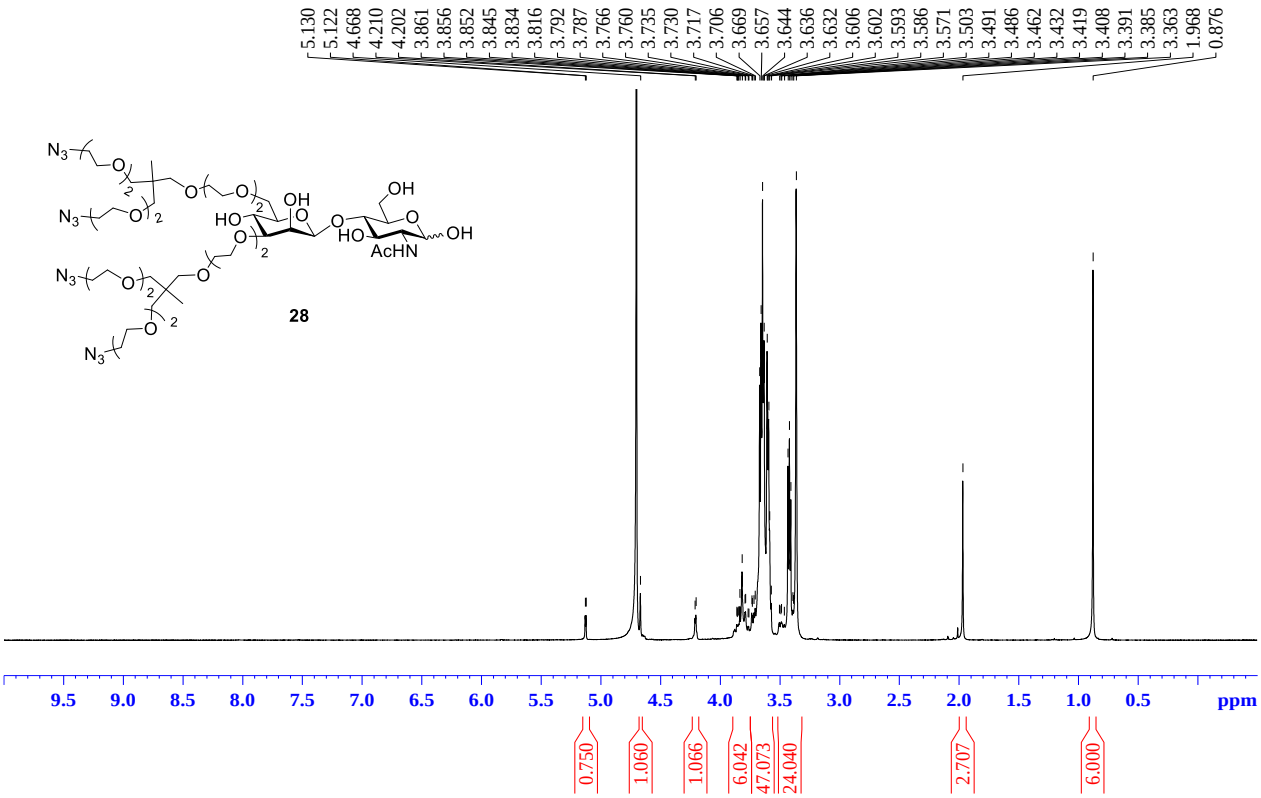
Compound 26: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



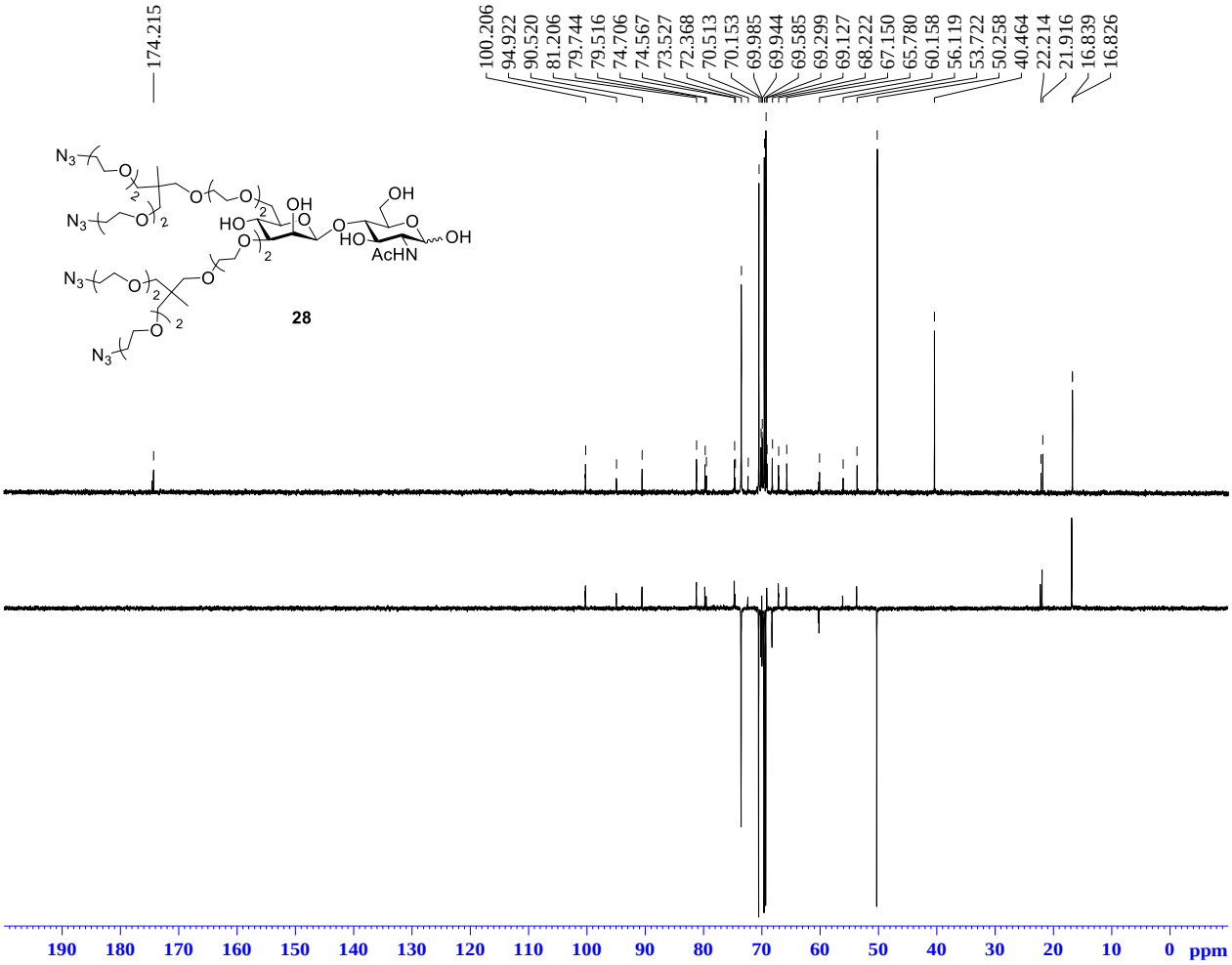
Compound 27: ^{1}H NMR ($CDCl_3$, 400 MHz)



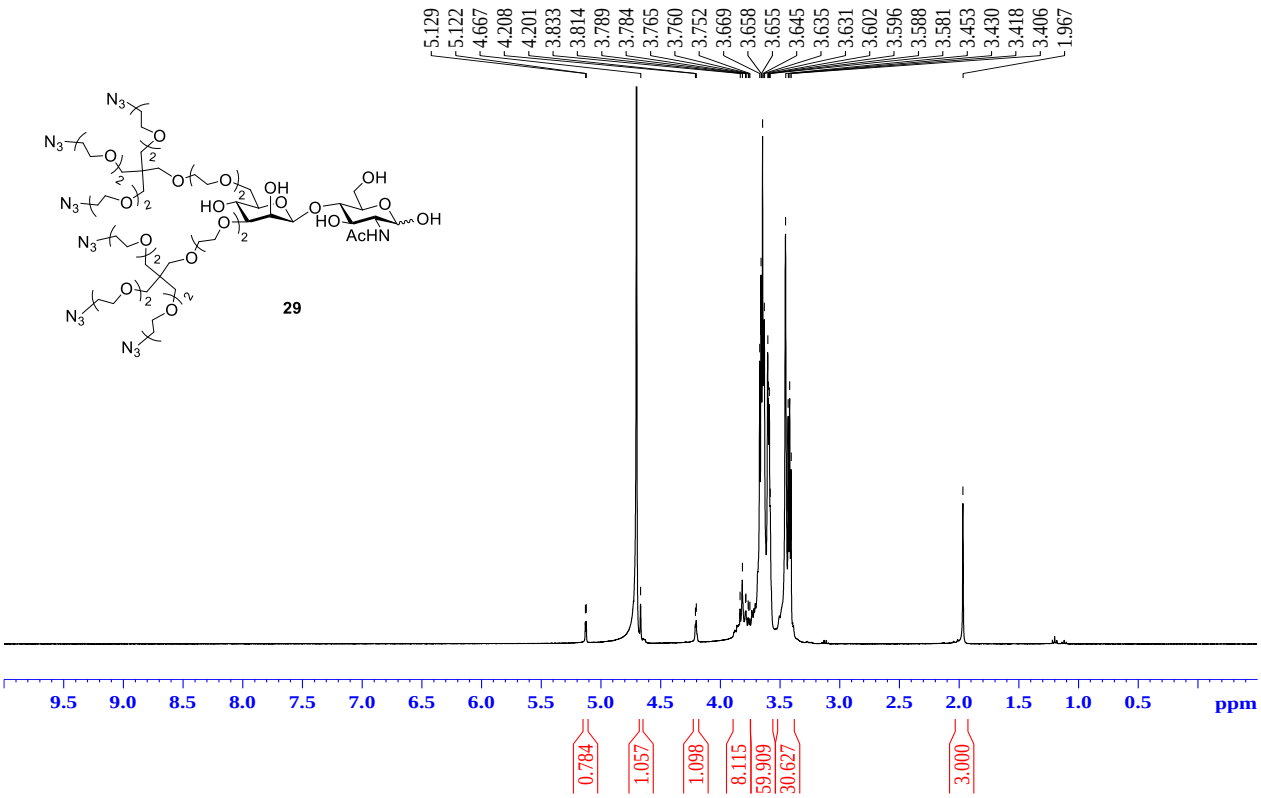
Compound 27: ^{13}C and Dept-135 NMR ($CDCl_3$, 100 MHz)



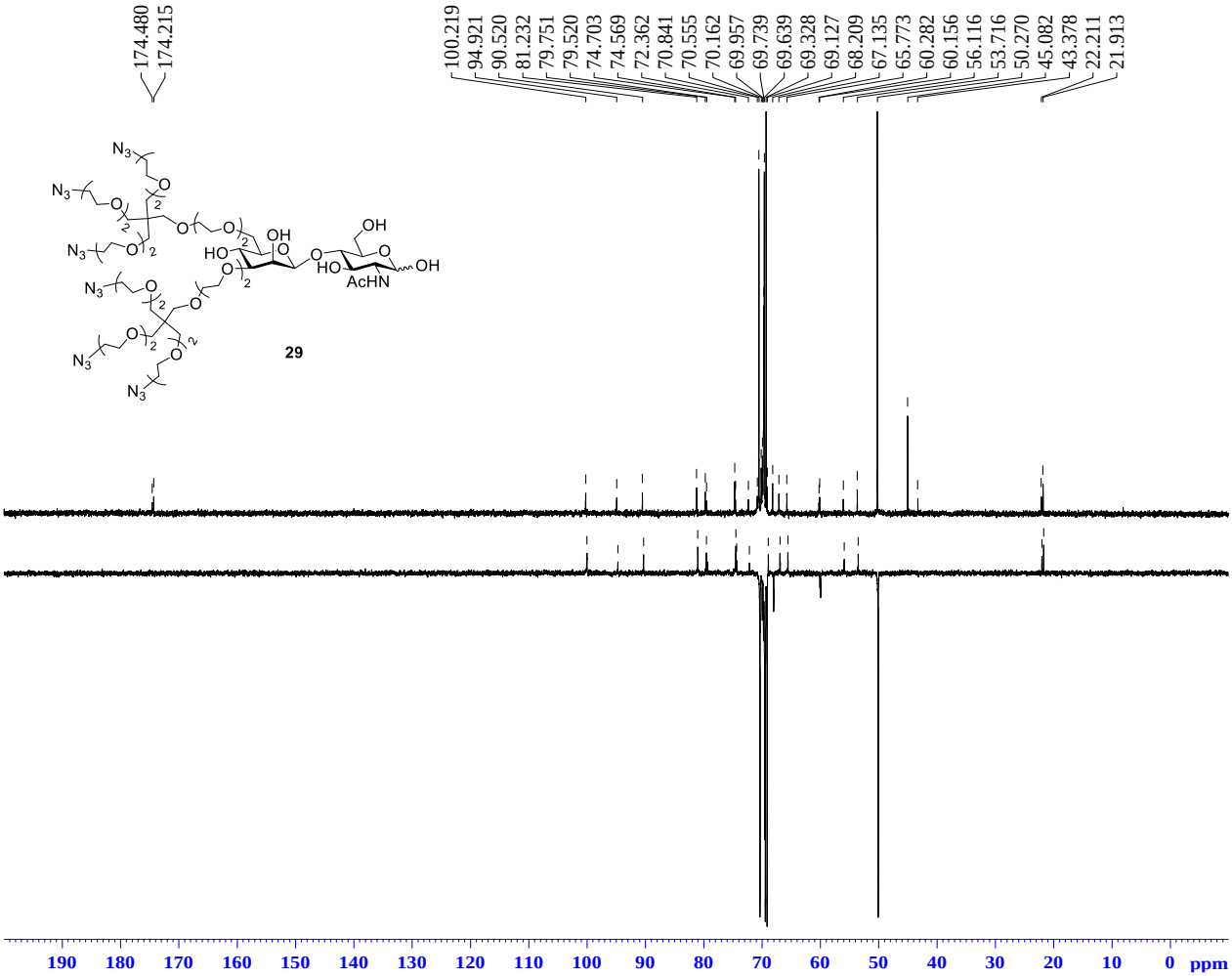
Compound 28: ^{1}H NMR (D_2O, 400 MHz)



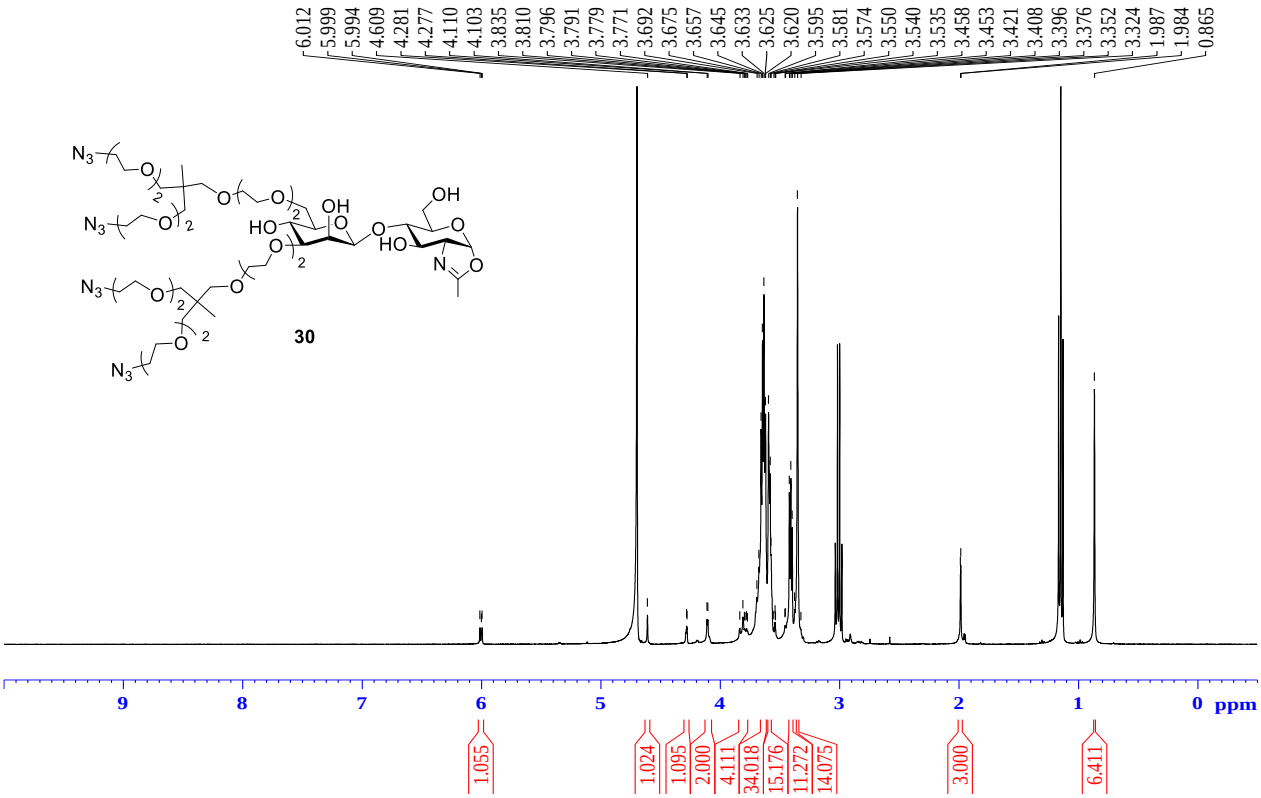
Compound 28: ^{13}C and Dept-135 NMR (D_2O, 100 MHz)



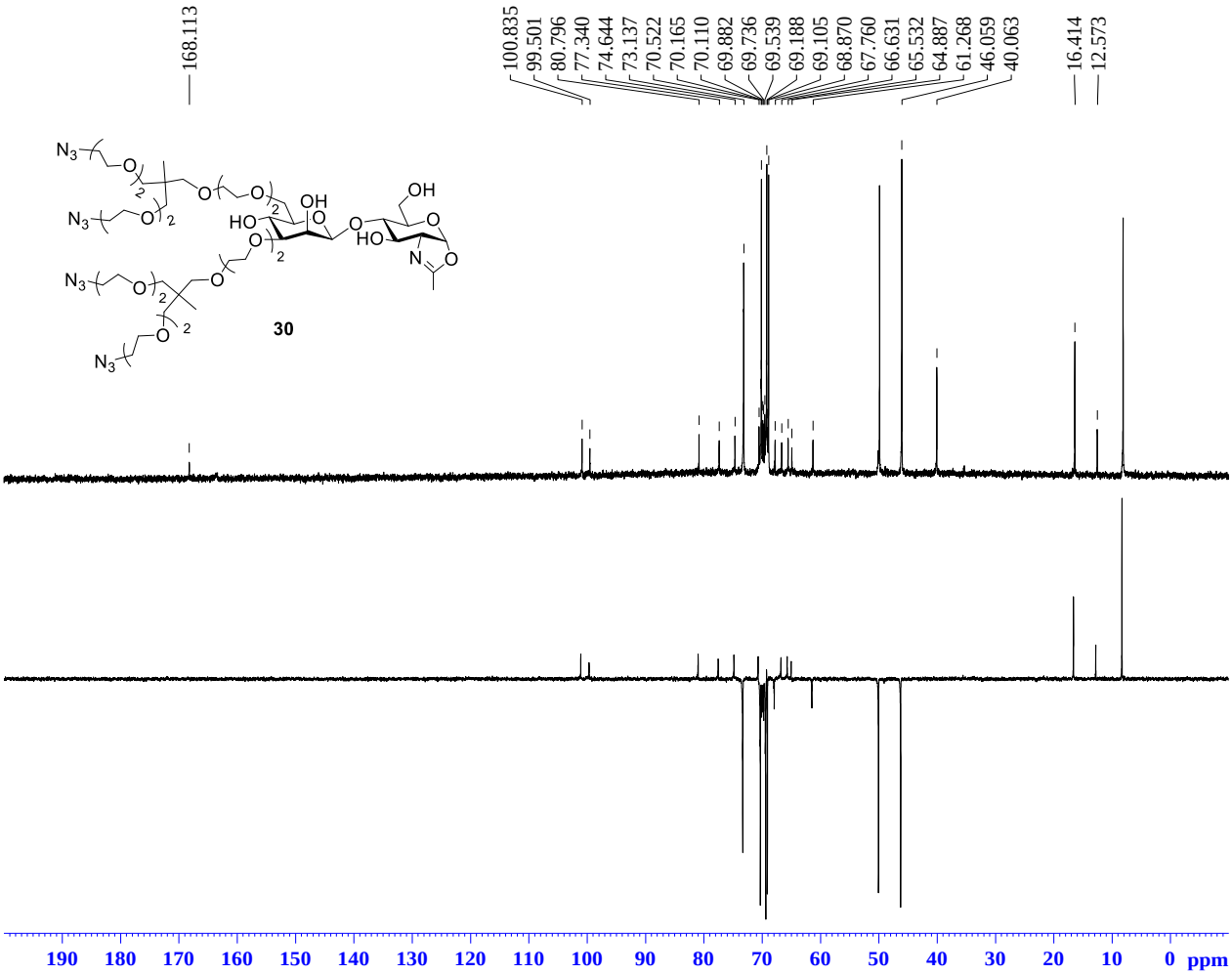
Compound 29: ^{1}H NMR (D_2O, 400 MHz)



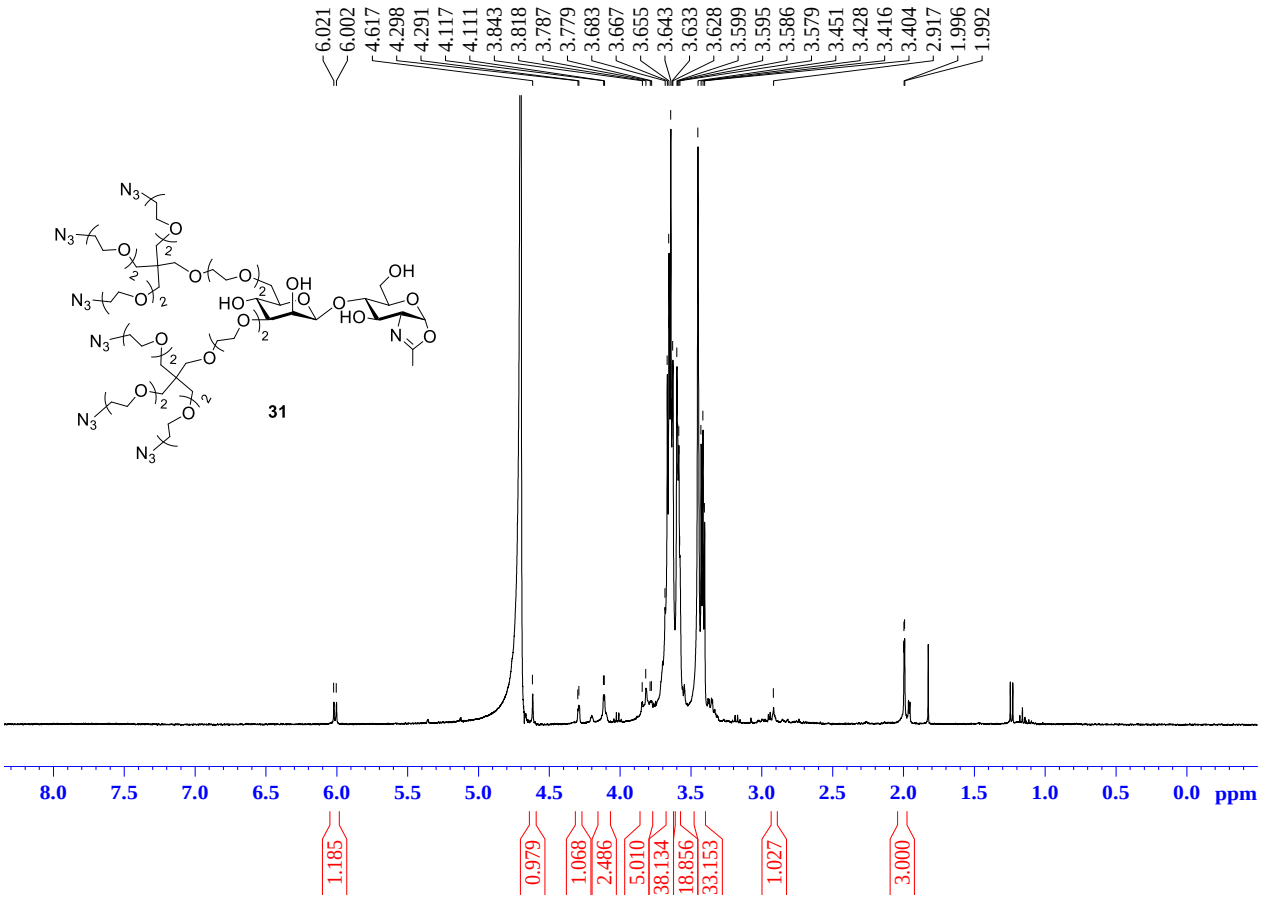
Compound 29: ^{13}C and Dept-135 NMR (D_2O, 100 MHz)



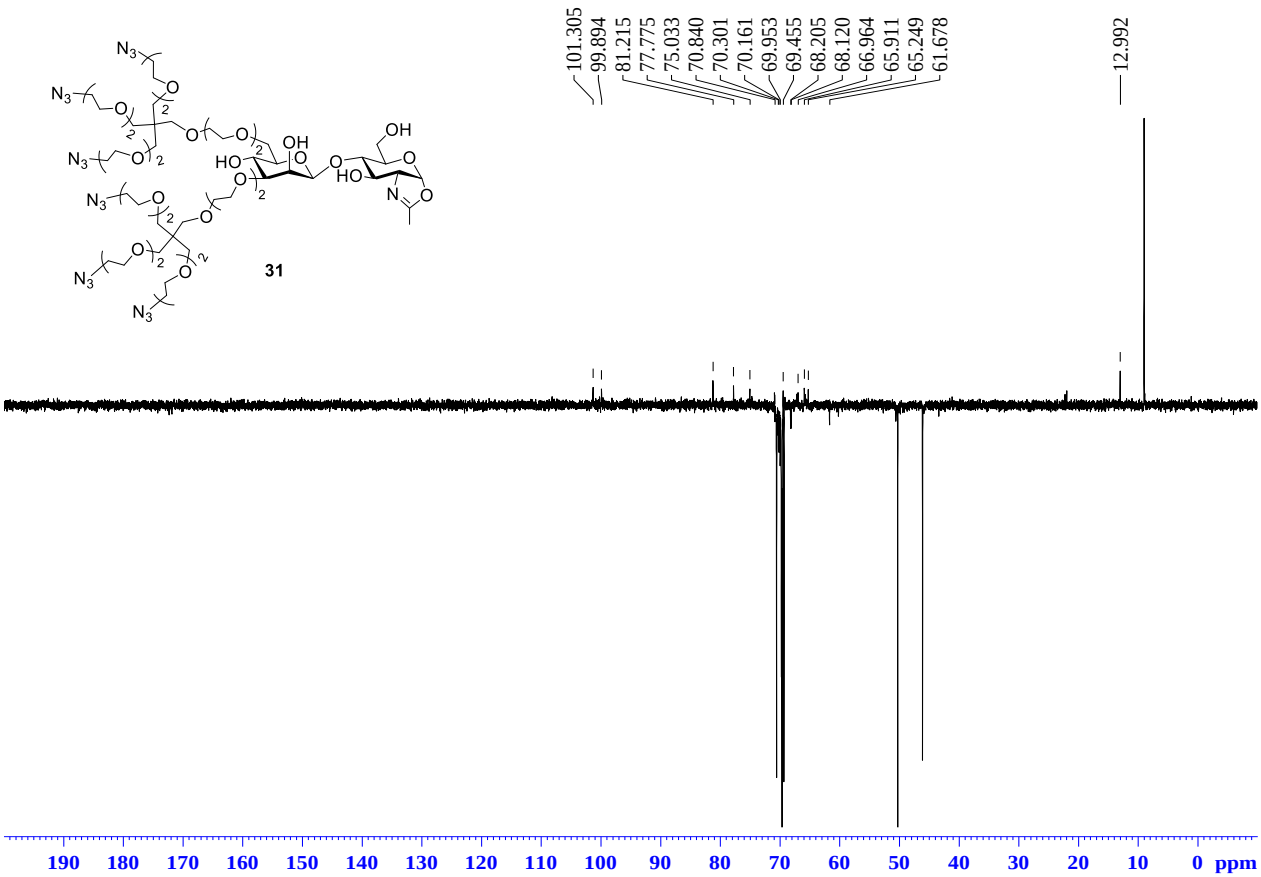
Compound 30: ^{1}H NMR (D_2O, 400 MHz)



Compound 30: ^{13}C and Dept-135 NMR (D_2O, 100 MHz)



Compound 31: ^{1}H NMR (D_2O, 400 MHz)



Compound 31: Dept-135 NMR (D_2O, 100 MHz)